ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 61. SUPPLEMENTUM 226

HUMAN SINGLE MUSCLE FIBER ACTION POTENTIALS

Extracellular recording during voluntary and chemical activation.
With some comments on end-plate physiology and on
the fiber arrangement of the motor unit

BY

JAN EKSTEDT

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AKADEMISK AVHANDLING

SOM MED VEDERBÖRLIGT TILLSTÅND AV
MEDICINSKA FAKULTETEN VID UPPSALA UNIVERSITET
FÖR VINNANDE AV MEDICINE DOKTORSGRAD
FRAMLÄGGES TILL OFFENTLIG GRANSKNING Å
FARMAKOLOGISKA INSTITUTIONENS FÖRELÄSNINGSSAL
LÖRDAGEN DEN 18 APRIL 1964 KL. 9 F.M.

av

JAN EKSTEDT
Med. lic., Smål.

UPPSALA 1964

ALMQVIST & WIKSELLS BOKTRYCKERI AB

ACTA PHYSIOLOGICA SCANDINAVICA

VOL. 61. SUPPLEMENTUM 226

FROM THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF UPPSALA, SWEDEN

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BOKTRYCKERI AKTIEBOLAG
UPPSALA 1964

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GENERAL INTRODUCTION

Electromyography, i.e. recording of the electrical activity accompanying muscular contraction, is an extensively used tool in the study of muscular physiology and has gained great importance in the diagnosis of neuromuscular disorders. References to previous work in the field of human electromyography can be found in the bibliographies of ROSENFALCK (1961, 1963). Comprehensive reviews concerning the present status of electromyography are, among others, those of BUCHTHAL (1962) and WOOLF (1962).

The electrical activity can be led off either by surface electrodes fastened to the skin overlying the muscle, or by intramuscular needle electrodes. Recording with surface electrodes can only give a very crude picture of the electrical activity, due to the considerable distance between the active fibers and the electrode. A needle electrode comes closer to the muscle fibers. Only the action potentials from the fibers within a limited area around the electrode are recordable. But at voluntary contraction the muscle fibers are not independently discharging; a number of muscle fibers—up to several hundred—are innervated by the same nerve fiber and constitute the motor unit. Therefore the individual muscle fiber action potentials are usually not discerned, but are mixed with other fiber action potentials from the same motor unit. The proportion in which each fiber takes part in the generation of this *motor unit potential* is dependent on where in the motor unit the recording is made.

BUCHTHAL, GULD & ROSENFALCK (1957 *a*) consider that the fibers of a motor unit in the human muscle are grouped in small bundles of, at most, 30 fibers; and these they term "subunits". The individual fiber action potentials in a subunit are considered to be highly synchronized and to give rise to "the spike of the motor unit potential"; that is, the "diphasic components of short duration and high amplitude" that are usually seen in the motor unit potential.

There is a vast literature concerning the motor unit potentials but very little has been written about single fiber potentials. Lately, some investigations of the membrane resting potentials in human muscle fibers *in situ,*

measured by capillary electrodes, have been published (JOHNS, 1960; NORRIS, 1962; CREUTZFELDT, 1962; RIECKER, BOLTE & VON BUBNOFF, 1963) and also recordings of the intracellular action potentials have been made (BERÁNEK, 1961). However, no study of human extracellular single muscle fiber action potentials *in situ* has been published. On the contrary, ADAMS, DENNY-BROWN & PEARSON (1962, page 117) in their monograph "Diseases of Muscle" state: "Attempts to record single fiber potentials by the use of electrodes with a very small 'pore', or active surface, in man have met with little success, for with active electrode tips below 50 μ in human muscle, movement artefact becomes magnified out of all proportion to the potential registered."

In the present paper a method of recording extracellular single muscle fiber action potentials in voluntarily activated human muscle is described. The method is based on the use of a needle multielectrode, where the electrodes can "look" at the action potential generator from different sites. Thus it is possible to select generators consisting of one single fiber.

The method of action potential pick-up and display are in many respects different from those used in ordinary electromyographic work and therefore they are described here in detail. The major part of this work deals, however, with the criteria to be fulfilled by single muscle fiber action potentials. The criteria have been deduced partly from the recordings of muscle fibers that were made to discharge spontaneously by chemical stimulation and partly from the study of composite motor unit potentials. It is shown that action potentials fulfilling these criteria must be derived from single muscle fibers.

From an analysis of the recordings has been concluded that the individual fiber potentials may also be discerned with ordinary concentric needle electrodes, and that the "spikes of the motor unit potential" are usually generated by single muscle fibers. This is contrary to the opinion of BUCHTHAL *et al.* (1957 *a*), who consider the spike source to be the "subunit" and only exceptionally a single fiber. The results obtained in this investigation are not consistent with the concept of a subunit arrangement of the fibers of a motor unit.

The present method of single fiber recording offers a new possibility for the study of the physiology of the neuromuscular junction. It is shown that the difference in the arrival time to the electrode of two fiber action potentials from the same motor unit is not constant from discharge to discharge. There are always small variations about the mean time interval, of an order of magnitude of 10–30 μsec, so that consecutive composite action potentials are of slightly different shape; the so-called *"action potential jitter"*. The jitter

phenomenon has been chiefly attributed to variations in the time delay in the neuromuscular junction. It does not seem to have been described in the literature. The absence of jitter has proved to be the most important criterion for single muscle fiber recordings.

With the multielectrode method the muscle fiber orientation and the direction of the propagation can also easily be determined, and this has been used here for delineation of the end-plate zone.

A short communication concerning single muscle fiber recording and propagation velocity determination has been published previously (Ekstedt & Stålberg, 1963).

SURVEY OF THE LITERATURE

Current concepts of the motor unit

The motor unit is considered as a physiological entity since the work of LIDDELL & SHERRINGTON (1925), who briefly defined it as "the motoneurone-axone and its adjunct muscle fibers".

According to most authors, all the muscle fibers of a motor unit are simultaneously active, but it has also been claimed that only a part of the fibers of a motor unit are active at the same time. RALSTON & LIBET (1953) consider that the degree of stretch in the muscle determines the number of active muscle fibers in a motor unit. This opinion has been neither supported nor refuted in subsequent literature. There is also the possibility that at fatigue there may be a partial neuromuscular block so that only part of the fibers are then active (BROWN & BURNS, 1949).

The number of muscle fibers per motor unit in different human muscles has been investigated by FEINSTEIN, LINDEGÅRD, NYMAN & WOHLFART (1955) and CHRISTENSEN (1959), who divided the total number of muscle fibers in a muscle with the total number of motor nerve fibers. Some examples of the values thus obtained are: first lumbrical 108, first dorsal interosseus 340, tibialis anterior 562, gastrocnemius 1934 (FEINSTEIN *et al.*); rectus superior oculi 23, opponens pollicis 13, brachial biceps 163, sartorius 300, rectus femoris 305, semitendinosus 713 (CHRISTENSEN).

However, the values found in these two investigations are based upon an assumption that 60 per cent of the large nerve fibers in all muscles are motor fibers, which, in turn, is based upon the investigations of the nerves to the cat anterior tibial and gastrocnemius muscles made by REXED & THERMAN (1948). The assumption was also supported by the findings in the nerve to a human gastrocnemius muscle with practically complete de-efferentiation due to poliomyelitis (FEX & WOHLFART (unpublished) quoted by FEINSTEIN *et al.*, 1955). It is, however, unlikely that the proportion of motor nerve fibers will be equal for such functionally different muscles as the gastrocnemius and the precision muscles of the hand or the external eye muscles.

The values given by both FEINSTEIN *et al.* (1955) and CHRISTENSEN (1959) are based upon one single determination for each muscle, except for a few where two muscles have been used. Therefore nothing can be said about the variations between different individuals. The calculations are also mean values for the respective muscle and nothing is known of the deviation about the mean in the individual muscle. Such a dispersion has been shown by ECCLES & SHERRINGTON (1930), who found considerable differences in contraction force between different motor units. They also found that in one and the same nerve the motor nerve fibers of large diameter gave rise to a more intense peripheral branching than did nerve fibers of lesser diameter, and this they considered an indication of the existence of motor units of different sizes. However, this latter opinion has been strongly opposed by FERNAND & YOUNG (1951) who consider that there is no correlation between nerve fiber diameter and the size of the motor unit. This conclusion has been drawn from comparisons between different muscles with a different mean size of the motor unit.

It is a well established fact that there is a certain order of recruitment of the motor units. At low contraction force there are only a few motor units active at a slow discharge rate. On increasing contraction, more and more motor units are recruited and the individual motor unit discharge rate is increased up to about 30–40 discharges/sec (BIGLAND & LIPPOLD, 1954).

Normally the motor units discharge entirely asynchronously but, especially during fatigue, there may be a tendency for a grouping of the motor unit potentials and this has been interpreted as a synchronization process between the moto-neurones (e.g. ADRIAN & BRONK, 1929; BUCHTHAL & MADSEN, 1950; BIGLAND & LIPPOLD, 1954; LIPPOLD, REDFEARN & VUČO, 1957). However, TAYLOR (1962) considers this grouping to be a purely chance appearance without biological significance and found no true tendency to synchronization of moto-neurone activity.

The motor unit potentials recruited at increasing contraction were found by KUGELBERG & SKOGLUND (1946 *a, b*) to be of higher voltage the greater the contraction force exerted. The order of recruitment of motor units was found to be the same at voluntary contraction and at electrical stimulation of the nerve by slowly rising currents. The motor units active at slight contraction were considered to be "smaller", i.e. to have less fibers than those recruited at higher contraction strength. These results also implied that "small" motor units should be innervated by large nerve fibers (having a lower threshold for electrical stimulation than smaller nerve fibers). This is contradictory to the findings of ECCLES & SHERRINGTON (1930), see above.

Buchthal, Erminio & Rosenfalck (1959) did not find any systematic difference in "territory" and "fiber density" (see below) in the motor units already active at slight contraction and in those that appeared at stronger contraction. This they interpreted as due to the motor units being of equal size.

The size distribution of the muscle fibers in different human muscles has been extensively studied by, among others, Schwalbe & Mayeda (1890). The diameters range from 10 to 100 μ. Smaller muscles, used in fine precision movements, e.g. the external eye muscles or the small muscles of the hand, have a smaller mean diameter than, for example, large muscles of the leg, used more for developing force. In the brachial biceps muscle—the muscle most used in the present investigation—Buchthal, Guld & Rosenfalck (1955 *a*) found a mean diameter of 48 μ with range 25–85 μ.

However, nothing is known about the size distribution within the individual motor unit, i.e. if there is the same size distribution within each motor unit, as in the whole muscle, or if there are motor units composed of predominantly small or large fibers.

It is probable that there is some sort of functional differentiation between different motor units, as shown in the cat (e.g. Granit, Henatsch & Steg, 1956) where there are "tonic" and "phasic" moto-neurones with clearly different properties. In animal muscle, differing function is also reflected in the muscle fiber structure (e.g. Hess, 1961); however, such differences have not been found in human muscle fibers (Coërs & Woolf, 1957). The question of the functional differentiation in human muscle seems, at present, to be open. A review of the problem has been presented by Ludin (1961).

It seems to be well established that the individual muscle fibers extend throughout the length of most muscles and that they are thus arranged in parallel (Feinstein *et al.*, 1955; Coërs & Woolf, 1957). The gracilis and sartorius muscles are exceptions, the fibers also being arranged in series.

The topographical arrangement of the fibers in a cross-section of the muscle has, however, been a matter of dispute. By dissection of the rabbit lumbrical muscle, Feindel (1954) found the fibers of a motor unit to be scattered, and separated by up to 8 fibers from other motor units.

By studying the atrophied muscle fibers after a limited experimental denervation, van Harreveld (1946) concluded that the fibers of a motor unit in the rabbit's sartorius muscle were widely scattered. Krnjević & Miledi (1958) stimulated single motor nerve fibers in a rat diaphragm preparation and found the muscle fibers innervated by that nerve fiber to be irregularly scattered over half the total area of the diaphragm.

Wohlfart (1959) considered there was an intermingling and a wide scattering of fibers from different motor units. These conclusions were based upon direct microscopic studies of the branching of nerves in rat muscle and on analyses of denervated muscles from mice with experimental poliomyelitis (Theiler virus). He writes: "Strikingly often only some of the fibers within the terminal groups of motor fibers were degenerating, while the remaining ones were intact. If a terminal motor bundle were part of only one motor unit, one would expect the entire bundle to be either degenerating or normal." This implies a reversal of his earlier position, i.e. that the muscle fibers of a motor unit were arranged in homogeneous bundles, based upon studies of the atrophy pattern in spinal paresis in man (Wohlfart, 1949).

Norris & Irwin (1961), using single nerve fiber stimulation and intracellular recording in the rat peroneus longus muscle, also found the fibers of a motor unit to be widely scattered. In the animal muscle, the intermingling and wide scattering of the fibers belonging to a certain motor unit seems thus to be well established.

In man, where more indirect methods of analysis have to be made, Buchthal, Guld & Rosenfalck (1957 *b*), Buchthal, Erminio & Rosenfalck (1959) have found the motor unit to be restricted to an approximately circular area, the "territory", with a diameter of the order of 5–10 mm. These conclusions have been founded upon electromyographic mapping of the cross-sectional area of the muscle by means of a multielectrode with the electrodes spread over a distance of about 20 mm along the shaft of the needle. The amplitude of the potential, obtained in this way from the center of the motor unit, was considered to be a measure of the fiber density of the motor unit. The product of the maximum amplitude and the area of the territory is therefore considered to be a measure of the number of fibers in the motor unit (Buchthal *et al.*, 1959). The territory was found to be enlarged in anterior horn cell disease (Erminio, Buchthal & Rosenfalck, 1959) and restricted in certain forms of myopathy (Buchthal, Rosenfalck & Erminio, 1960).

Do Carmo (1960) investigated the territory of the motor unit in newborn infants using the multielectrode method of Buchthal *et al.* and found approximately the same diameter of the territory as in adults, in spite of the considerably lower total cross-sectional area of the muscles, and in spite of the same number of motor units and muscle fibers. At present no explanation is forthcoming that accounts for this interesting finding.

Buchthal *et al.* (1957 *a*) claim that the motor unit is subdivided, the muscle fibers being grouped in bundles of 10–30 adjacent fibers, termed "subunits", approximately evenly distributed within the territory of the motor

unit. The subunit gives rise to "the spike of the motor unit potential", i.e. a smooth clean spike potential of high voltage and short duration. These conclusions have been founded on the following data:

1. The spike potential was recorded with a multielectrode having 14 small electrodes spread over a distance of 2.5 mm in an opening at the side of a needle. Spikes of high amplitude (more than 5 mV) were considered to have been obtained with the electrode positioned just outside the spike source. Assuming an exponential voltage decrement with increasing distance, the distance between the needle and the center of the spike source was calculated to be 85–150 μ, and this was considered to be the radius of the spike source. With a mean fiber diameter of 56 μ, the spike source (the "subunit") was then calculated to contain 10–30 adjacent fibers. No fiber from other motor units was assumed to be within the subunit.

The smooth, clean shape of the subunit spike, in spite of its being generated by 10–30 fibers of different diameters and with different sites of innervation, was explained as an effect of a mechanism of mutual interaction between adjacent fibers which equalized the propagation of the individual fiber action potentials.

2. Sometimes two spike sources were found within the area scanned by the multielectrode. The mean distance between the center of each pair of spike sources was found to be 0.7 mm (0.3–1.3 mm, 15 recordings). Assuming an even distribution of the spike sources at a mutual distance of 0.7 mm within a territory of the motor unit of 5 mm (BUCHTHAL *et al.*, 1957 *b*) and assuming the motor unit to contain 1000 muscle fibers, each spike source was calculated to contain 19 muscle fibers; this is of the same order of magnitude as found above.

3. In spinal paresis due to amyotrophic lateral sclerosis an analysis of muscle biopsies reveals groups of 10–50 fibers with about the same degree of atrophy (WOHLFART, 1949). Buchthal *et al.* have repeated this histological investigation in mild cases of amyotrophic lateral sclerosis and found 10.2 ± 6.7 fibers per atrophic field. The atrophic fiber groups were assumed to be "subunits".

In later reviews of the motor unit organisation, BUCHTHAL (1961, 1962) refers to the subunit as containing "1–30 fibers".

No evidence for a subunit arrangement has been found in the animal experiments, mentioned above, by VAN HARREVELD (1946), FEINDEL (1954), KRNJEVIĆ & MILEDI (1958), NORRIS & IRWIN (1961).

An important factor when considering the possibility of recording single muscle fiber action potentials is also the degree of simultaneity between the different fibers of a motor unit. A certain measure of this is the duration

of the motor unit potential. In the biceps muscle PETERSÉN & KUGELBERG (1949) and BUCHTHAL, PINELLI & ROSENFALCK (1954 *b*) found mean values of duration of the motor unit potentials of about 7 msec, with extreme limits of 3 and 22 msec. With a duration of a single muscle fiber action potential of less than 1 msec (page 72) it can be concluded that the action potential from a certain fiber is not coincident in time with more than a part of the motor unit fiber potentials.

The duration of the motor unit potentials is due to differences in the arrival times of the individual fiber action potentials to the site of recording. These differences have been attributed by BUCHTHAL *et al.* (1955 *b*) mainly to the different sites of initiation of the impulses due to the extent of the innervation zone, and this was found to be about 40 mm in the biceps muscle for the same motor unit. They do not consider different propagation velocities of the muscle fibers and different propagation times in the terminal nerve endings to be important factors for the duration of the motor unit potential.

The topography of the terminal innervation of muscle has been extensively studied by COËRS & WOOLF (1957) and CHRISTENSEN (1959). The end-plate was located in the center of the muscle fiber (except for the long muscles, sartorius and gracilis, which have a scattered innervation) and there was generally only one end-plate per muscle fiber. There was a double innervation in 2.3 per cent of the muscle fibers, but it was never observed that one muscle fiber was innervated from two different axons (COËRS & WOOLF, 1957). Also physiological investigations provide no basis for a polyneuronal innervation (BROWN & MATTHEWS, 1960).

In man there seems to be a rather uniform shape of the end-plates, both when comparing them in one and the same muscle and in different muscles. There seems to be a correlation between the size of the end-plate and diameter of the muscle fiber. The mean diameter of the end-plates in a series of human limb muscles was found to be 32.2 ± 10.5 μ, with a range of 10–80 μ (COËRS, 1955; COËRS & WOOLF, 1957).

Different types of electrodes used in human electromyography

Since ADRIAN & BRONK (1929) introduced the concentric needle electrode this has been the most frequently used type of intramuscular electrode for routine electromyography. It consists of an ordinary injection needle with a centrally mounted platinum wire exposed at the bevel point. The diameter of the central wire is often about 100 μ and the exposed surface has an oval

shape. The outer diameter may vary between 0.35 and 0.65 mm for the electrodes that are commercially available. Recording is made between the center core and the outer needle. The physical parameters of concentric needle electrodes have been investigated by BUCHTHAL, GULD & ROSENFALCK (1954 *a*).

Instead of one, two center cores may be used and, by bipolar recording between these, the area of uptake may be considerably restricted and the electrode accordingly made more sensitive in picking up potentials only from fibers in the immediate vicinity of the tip of the electrode (e.g. LANDAU, 1951 *b*).

Another commonly used type of electrode is a sewing needle or an entomological needle of outer diameter 0.3–0.5 mm, lacquered except for the extreme tip. The bare tip has a diameter of 25–50 μ. These electrodes are easy to construct and give reliable results, but are prone to develop small cracks in the insulation, creating false leading-off surfaces that can give rise to a misinterpretation of the electromyogram. This type of electrode has been used extensively, e.g. by JASPER & BALLEM (1949), and in the extraocular muscles by BJÖRK & KUGELBERG (1953 *a, b*).

When electromyography is used for the physiological study of movement or of discharge patterns of the motor unit, a very practical type of electrode consists of two fine insulated wires, e.g. of tungsten, introduced into the muscle by an injection needle that is subsequently withdrawn. As the wires can be made very fine, they can be kept in place in the muscle for a long time without the subject feeling their presence. However, once inserted, their position can only be altered by retracting through the insertion channel created by the needle, where the muscle fibers are damaged. This electrode type is not suited for the study of the action potential shape. It has been used by BASMAIJAN (1962) in determining the particular muscles that are concerned in specific movements (kinesiology) and, further, by BIGLAND & LIPPOLD (1954) and LIPPOLD, REDFEARN & VUČO (1957) in studies of the motor unit discharge pattern. A technical description of such an electrode is given by BASMAIJAN & STECKO (1962).

The type of electrode has a great influence on the shape of the action potentials recorded. Comparative investigations concerning this have been made by PETERSÉN & KUGELBERG (1949), PINELLI (1949), LANDAU (1951 *a*), LUNDERVOLD & LI (1953) and BUCHTHAL *et al.* (1954 *a*).

An entirely new design of composite electromyographic electrode was introduced by BUCHTHAL *et al.* (1957 *a*), the needle multielectrode. At the side of an injection cannula up to 14 electrodes were mounted. Different multielectrode types were constructed. One type had fourteen 50–100 μ leads arranged in a row of total length 2.5 mm, and was used for the study

of the volume conduction of the spike of the motor unit potentials. Other types had greater leading-off areas and longer mutual distances for the study of the territory of the motor unit (BUCHTHAL *et al.*, 1957 *b*). The great advantage of this type of electrode is that the action potential from one and the same generator can be led off from accurately known positions in a section of the muscle. This type of multielectrode was used as the basic consideration in the design of the multielectrode used in the present investigation.

A needle electrode design with one or several electrodes mounted in an opening at the side of a needle has been used by FLECK (1962). He has also introduced a type of needle electrode with electrodes mounted in two openings at opposite sides of a needle. This type has been used in this investigation as well (page 19).

Glass capillary electrodes, introduced through an injection cannula, or an incision through the skin, have been used for measurements of the muscle membrane resting potential by JOHNS (1960), NORRIS (1962), CREUTZFELDT (1962) and RIECKER, BOLTE & VON BUBNOFF (1963). Only BERÁNEK (1961) seems, however, to have published any recordings of the intracellular action potential at voluntary contraction using such techniques. After having inserted an injection cannula, one of the fibers, pressed into the opening of the bevel point by the intramuscular pressure and thus made immobile, was pierced by a glass capillary electrode that was slowly advanced within the needle by a micromanipulator.

Still another type of intramuscular electrode is the "electromyographic myotome" (BONSETT, ABREU & RALSTON, 1961), with which it is possible to take a muscle biopsy from the specific region where the electrical recording has been made.

CHAPTER 1

METHODS

Recording

Electrodes

The 3 + 11 multielectrode

In an opening (0.5 × 1.5 mm) at the side of a stainless steel injection cannula 14 small round electrodes were arranged (Fig. 1). The electrodes were the cut ends of 30 μ platinum wires, each connected to one pin of a multipolar plug, directly mounted on the needle. The wires were embedded in heat-hardened epoxy resin (Araldite®, Ciba, Basle, Switzerland) and ground flush with the side of the needle. The electrodes were arranged in two rows, one with 3 and the other with 11 electrodes. The distance between the two rows was 200 μ and the adjacent electrodes were about 60 μ apart. The electrodes were thus spread over a distance of 600 μ along the needle. The face of the array and the adjoining part of the needle were ground flat. Thus, the needle had two diameters, 0.5 and 0.55 mm.

A small needle diameter was chosen so that the least damage was done to the muscle fibers upon insertion. The condition of the point of the needle was found to be of fundamental importance. It was confirmed many times that when the point was even slightly bent or was blunt it was very difficult to obtain action potentials of high amplitude, probably due to damage to fibers close to the needle. Therefore the point was reground under the microscope before each experiment, first, with number 3/0 emery polishing paper and, then, by strapping against smooth leather. The bevel surface was at the opposite side of the needle from the electrode array, so that those fibers pushed aside by the bevel point, and perhaps compressed, were not used for recording. The subject was grounded by the shaft of the multielectrode. No difference in the shape of the recorded action potentials could be detected when shifting from this way of grounding to a large skin electrode with the shaft disconnected from ground.

The leads from the electrodes run close together for a length of 5 cm within the needle. This fact was responsible for an inter-electrode capacitance of 8–10 $\mu\mu$F (page 29) for the different electrode combinations. The

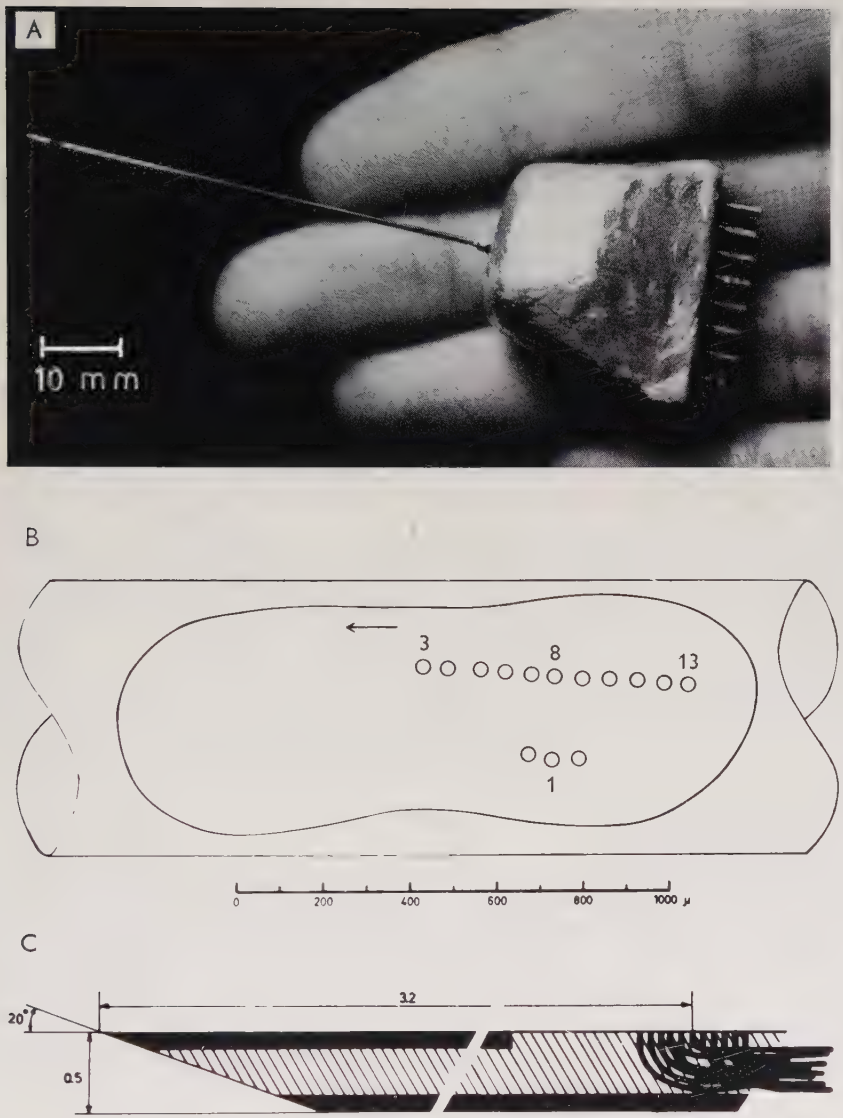

FIG. 1. *The 3+11 Multielectrode. A,* Photograph of the multielectrode. *B,* The region with the electrode array magnified about 550 times; redrawn from microphotographs. *C,* Schematic, longitudinal section through the long electrode row.

inter-electrode capacitance was further increased by 5–10 $\mu\mu$F in the input cable of the amplifiers (page 28). This gave rise to a capacitive cross-talk of the signals between the different electrode leads. The amount of charge transferred through a capacitive coupling is proportional to the rate of rise

of the voltage difference. The cross-talked signal between two electrode leads was usually of the order of 4 per cent (see Fig. 36). The cross-talk was responsible for the inequalities in the action potentials from electrodes 1 and 8 seen in some of the recordings (e.g. Fig. 10). Cross-talk is discussed further on page 75.

Not only the cross-talk, but also the noise level and the shunting of the higher frequencies by stray capacitances to ground, are functions of the electrode impedance, which must therefore be kept low. It can be substantially reduced by a method of electrolytic treatment of the electrodes, devised by BUCHTHAL *et al.* (1957 *a*). Their method has been adapted in this work and was performed in the following way prior to each experiment. The multielectrode was immersed in physiological saline. The negative pole of a 4.5 volt battery was connected to one of the electrodes via a 0.1 megohm resistor. The other pole was connected to a platinum needle, also immersed in the saline bath. The current, 10–20 μA, was passed through the electrodes for 10–20 seconds, under microscopic control. The procedure was repeated for each of the electrodes.

The electrical properties of the multielectrode are further discussed on page 29.

The positions and the distances between the centers of the electrodes were determined by microphotography. A stage micrometer with 10 μ divisions (J. D. Moller Wedel in Holstein) was photographed at the same magnification and used for measurement at a total magnification of 2000 times. The maximum error in the measurements was estimated to be less than ± 2 μ.

The electrodes were not positioned in the strict geometrical pattern that was originally intended. The inter-electrode distances varied between 52 and 63 μ. The greatest differences happened to be between the electrode combinations 7–8 and 8–9, which made these electrodes unsuitable for the special bipolar recordings (page 39). For these recordings electrode combinations 8–9 and 9–10 with the distances 61 and 63 μ, respectively, were used instead. In all calculations and measurements actual distances were always used.

The long electrode row happened to deviate 8° from the long axis of the needle, and this had to be compensated for when making rotation adjustments of the needle position.

Damage to the electrodes or the surrounding insulator sometimes made re-polishment of the needle necessary. If extensive, such a re-polishing could change the interelectrode position so that new microphotographic measurements were necessary.

The needle was sterilized in boiling water for 20 minutes.

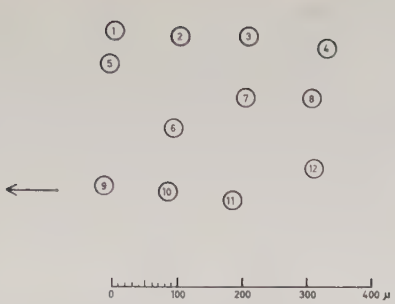

FIG. 2. *The 3 × 4 Multielectrode.* The electrode arrangement in the opening at the side of the needle; redrawn from a microphotograph. The arrow points towards the tip of the needle. The general appearence of this multielectrode is the same as for the 3 + 11 multielectrode shown in Fig. 1.

The 3 × 4 multielectrode

Due to a constructional failure the electrode arrangement in this multielectrode became very irregular, but just this property made it useful for some special applications. There were three irregular rows with four round platinum electrodes of diameter 30 μ (Fig. 2) in an opening at the side of a needle with diameter 0.55 mm. The constructional and electrical properties were the same as for the 3 + 11 multielectrode.

The "Janus electrode"

This electrode (Fig. 3) was pointing in two opposite directions simultaneously, and has accordingly been designated a "Janus electrode". One platinum electrode, of diameter 25 μ, was mounted centrally in each of two openings at the opposite sides of a needle, 0.60 mm in diameter. This needle electrode was used for making simultaneous recordings from both sides of the needle, the objective being to elucidate some problems concerning the motor unit organisation.

A similar electrode has been described by FLECK (1962).

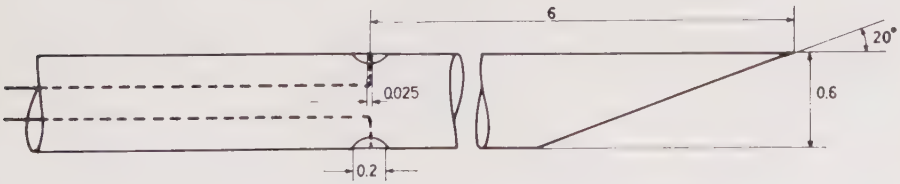

FIG. 3. *The "Janus electrode".* Two round platinum electrodes, mounted opposite to each other flush with the shaft of the needle.

The concentric needle electrodes

The concentric needle electrodes used were types 13 K 51 and 13 K 03 (Disa Elektronik, Herslev, Denmark). The center core was of diameter 100 μ, the needle diameter 0.45 mm (13 K 51) or 0.65 mm (13 K 03) and the bevel angle 15°.

The reference electrode

Recording was usually made between the electrodes in the multielectrode and a subcutaneous needle electrode as a reference. The latter had a platinum surface of area 0.05 mm^2 exposed in the bevel surface of an injection needle.

To make the reference electrode less liable to pick up activity from the muscle under investigation it was placed over the muscles antagonistic to it. That the reference electrode was electrically indifferent was always checked at the time of the experiment.

The cross-talk could be considerably reduced by using one of the electrodes in the multielectrode as reference (electrode 3 or 13 in the 3 + 11 multielectrode), as used by BUCHTHAL *et al.* (1957 *a*). Approximately similar cross-talked signals were then fed to both grids of the differential amplifier and cancelled or, at least, partially so. However, it was necessary to ascertain at each individual recording that the reference electrode did not pick up any activity (see BUCHTHAL *et al.*, 1957 *a*), and this proved to be tedious and time-consuming. Therefore the subcutaneous reference has usually been preferred.

Amplifiers

Each of the 14 electrodes in the multielectrode had its own cathode follower (capacity coupled) and the leads were surrounded by individual screens connected to the cathodes of the cathode followers (see ATTREE, 1949; NASTUK & HODGKIN, 1950). This arrangement, with a multiplicity of cathode followers, was chosen instead of using selectors for both lead and screen in the high impedance input circuits (BUCHTHAL *et al.*, 1957 *a*) as the wiring to the selectors inevitably introduced a considerable capacitance both to ground and between the leads. This affected the high frequency response and increased the cross-talk between the leads when using high impedance electrodes. By means of six selectors each cathode follower output could be connected to any of the input grids of three differential amplifiers.

The drawback with this arrangement was, however, the need for ac-

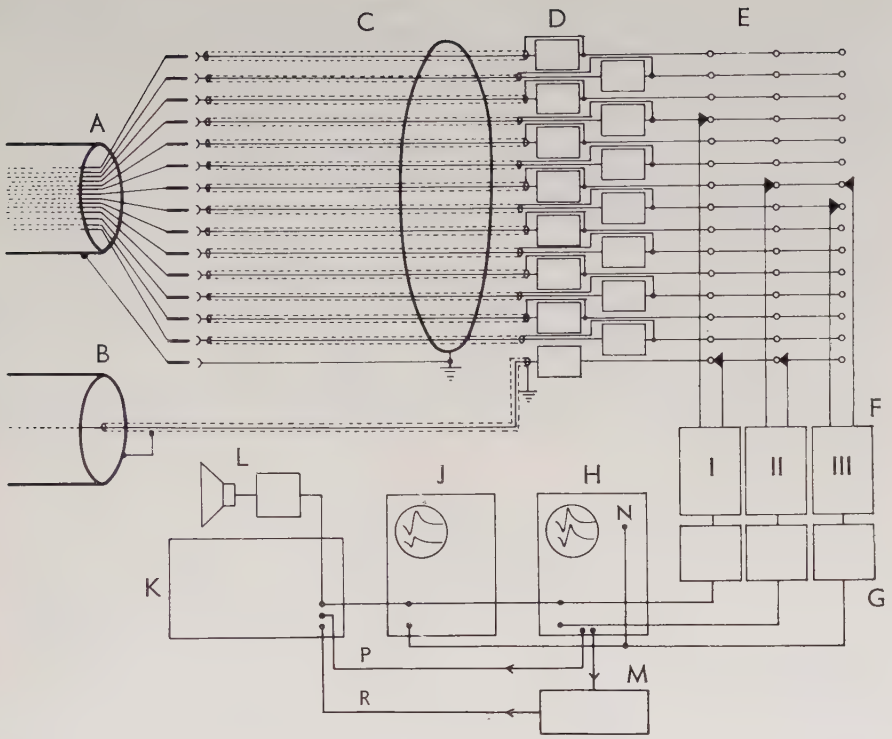

FIG. 4. *Block diagram of the input circuitry and the experimental set-up. A,* Multielectrode. *B,* Reference electrode. *C,* Input cable with separate screening for each lead. *D,* Input cathode followers with their outputs connected to the screens of the input leads. *E,* Selectors. *F,* Differential amplifiers. *G,* Attenuators. *H,* Oscilloscope used in the photographing recording. *J,* Monitoring oscilloscope. *K,* Ink-jet recorder. *L,* Loudspeaker with amplifier. *M,* Photography device, governed by the oscilloscope. *N,* External trigger input from channel III. *P,* Signals marking the sweep of the oscilloscope. *R,* Signals marking the period during which the camera shutter is open.

curately matched valves and other components. By careful selection and adjustments it was possible to equalize the gain of the different cathode followers to within 0.15 per cent, which corresponded to a common-mode rejection of about 700 times (see page 29).

The 14 individually screened input leads had a common, grounded screen. The multicable, of length 70 cm, was flexible and light and had an outer diameter of 9 mm (Disa Elektronik, Herslev, Denmark).

The two amplifiers that were used for recording had identical properties to within 1 per cent. They were capacity coupled without feedback. The preamplifier parts were similar in construction to the Tetronix type 122

Low-Level Pre-amplifier. Attenuators made the amplification variable between 0.3 μV and 10 mV/cm on the oscilloscope in 22 steps with factor $\sqrt{2}$. The attenuation was frequency independent with an accuracy better than 1 per cent.

The bandpass was variable stepwise between 0.2 and 40,000 cps (– 3 db). It was usually set at 60–40,000 cps (limited to 35,000 cps by the multielectrode; see page 29).

The signals from two electrodes could be added in the amplifiers (see page 44).

The specifications for the amplifiers are given on page 28.

The third differential amplifier had a limited frequency response but was only used for monitoring and triggering purposes.

A block diagram of the input electronic circuitry and the experimental set-up is given in Fig. 4.

Action potential display

In ordinary electromyography the potential sampling for the study of action potential shape is usually a purely random procedure. In the diagnosis of, for example, myopathy, where the pathological processes are known to be patchy within the muscle, it may be necessary to search for a pathological area (KUGELBERG, 1949), but once action potentials are picked up no adjustment of the needle for any "optimum position" within the motor unit is made. The action potentials are usually displayed with free running sweep on the oscilloscope and photographed with the film slowly moving perpendicularly to the sweep direction (see e.g. BUCHTHAL, 1957). The action potentials appear randomly at the different sweeps but can easily be identified for measurement.

The present situation was, however, entirely different. It was necessary to search for a certain position in relation to a muscle fiber and to keep the multielectrode in that position with high precision during the whole recording procedure. It was also necessary to check the single muscle fiber criteria directly on the oscilloscope before recording. This was not feasible with the action potentials randomly occurring at different sites on the oscilloscope screen, usually together with several action potentials from other motor units. The only possible way seemed to be to trigger the sweep by the initial positive phase of the action potentials, as performed by BUCHTHAL *et al.* (1957 *a*). Then all consecutive action potentials were displayed at the same position on the screen and were seen as a fixed pattern even at a discharge rate of only a few action potentials per second.

When the initial positive phase of an action potential reached an optional

voltage the sweep triggered. If several different action potentials were picked up by the electrode, all of them that had a positive phase reaching the particular triggering level were displayed. In order to select only one action potential, the triggering level must be increased until only that action potential with the biggest initial phase remains on the screen. If two action potentials had positive phases of the same voltage they could be separated by triggering the sweep, not at a certain voltage but at a certain change of voltage, i.e. the first time derivative of the voltage.

Another possible way to separate two action potentials with about the same positive phase was to use the signals from another electrode for triggering, where the action potentials from the two sources markedly differed in amplitude. In general, however, the procedure of triggering favoured the action potentials of high amplitude if high and low amplitude potentials were picked up at the same time.

A Tectronix (Beaverton, Oregon, U.S.A.) dual beam oscilloscope, type 502 with P 11 phosphor, was used for recording. A sweep of 20 or 100 μsec/cm was usually used. To facilitate the identification of the two channels one of the beams could be intensity modulated by a $5–20 \times 10^4$ cps A.C. signal.

A second dual beam oscilloscope (Telequipment, type D 31, London, Great Britain) was used as an aid for keeping the multielectrode in position while photographing the above-mentioned oscilloscope.

The screen was photographed by a 16 mm movie camera (Beaulieau R 16, Paris, France). A special device made the camera advance one frame when a sweep had passed on the oscilloscope and kept the shutter open until the next sweep had passed, and so on. The camera thus followed the action potentials, recording all up to a discharge rate of 4/sec. At higher rates the camera photographed the first discharge to come after the "refractory period" of the camera device. At a discharge rate of 20/sec, every fifth action potential was thus photographed, one on each frame. For comparison of the shape of consecutive discharges of an action potential, an optional number of discharges could be superimposed on one frame (see e.g. Fig. 19). By altering the vertical position of the trace a few millimeters for each discharge, a "carpet" of action potentials was obtained where the variations in time interval between two components could be conveniently studied (see Fig. 23).

The number of the exposure and the time of exposure were indicated on electromagnetic counters which were also recorded on each frame. By using a fast, but nevertheless extremely fine grain, negative film (Eastman Double X, type 7222, Rochester, N.Y., U.S.A.) developed in Kodak D 76 developer for 12 minutes, the beam intensity of the oscilloscope could be kept low. In

spite of the small size of the image no loss of detail was noticed when it was projected for measurement at a magnification of 27 times, corresponding to 1.5 times the actual size. For this purpose a 16 mm film editor viewer was used (model Luxe, Muray, Paris, France), which was modified by a larger hood and screen thereby producing greater magnification than the original and improving the linearity of the projected image. The oscilloscope graticule was photographed on each frame and used as reference for the measurements. The procedure of photographing and projecting therefore did not increase the uncertainty in the measurements (cf. page 29). By cinematographic projection of the film in the viewer, comparison of the shape of consecutive action potentials could be made in the same way as when directly inspecting the oscilloscope.

For the study of the innervation frequency and rhythm, the action potentials were also recorded on one channel of a three channel ink-jet recorder with a paper speed 1–10 cm/sec (Mingograph type 24 B, Elema, Stockholm, Sweden). Owing to the limited high frequency response of the recorder (600 cps) the action potentials were seriously distorted, which was, however, not important for this application. On the second channel, each sweep of the Tectronix model 502 oscilloscope was signalled, and, on the third channel, there was a timing mark and a signal during the period when the camera shutter was open (Fig. 5). Thus, the same action potential could easily be identified on the oscilloscope photograph and on the ink recording.

Loudspeaker display was invariably used as an indispensable aid in the positioning of the multielectrode and in monitoring the recordings.

Calibration

For voltage calibration the built in 10 mV calibration pulse in the Tectronix type 502 oscilloscope was used. This, in turn, was calibrated to within 1 per cent absolute accuracy with a model 44 Substandard Multi-Range Meter (Electronic Instruments Ltd., Great Britain).

A Philips (Einthoven, Holland) Gated Timing unit, Gm 8892/09, was used for time calibration. This delivered pulses at intervals of 100 μsec with an accuracy of 0.1 per cent.

The voltage and time calibrations were checked before each experiment, but had to be reset only exceptionally.

General experimental procedures

The detailed procedures will be dealt with in Chapter 2 in conjunction with the discussion of the single muscle fiber action potentials. The following general remarks apply to all the special procedures to be described later.

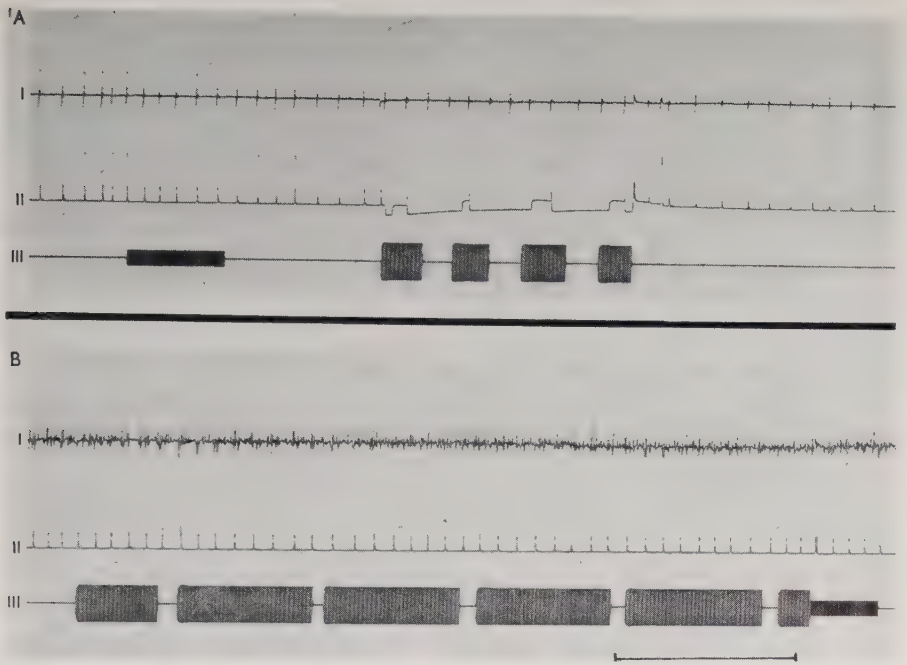

FIG. 5. *Examples of ink-recordings.* Channel I records the action potentials. Channel II records the instant when the sweep is triggered on the oscilloscope and channel III the period during which the camera shutter is open (broad markings). The narrow markings appear every 10th second. In the recording *A*, single sweeps have been photographed on the oscilloscope. In the recording *B*, two to eight consecutive action potentials have been superimposed on each oscilloscopic photograph.

Note in *B* how the oscilloscope selects the action potentials from only one of the several sources that are active at the same time. Calibration: 1 sec.

The electrode was usually inserted with the muscle under slight contraction and with the plane of the cutting edges of the tip parallel with the direction of the fibers. This was considered to cause as little damage as possible. When searching for action potentials an amplification corresponding to 50–100 μV/mm was used. When a suitable action potential was found, the amplification was adjusted so that the amplitude registered 3–6 cm on the oscilloscope. A sweep of 100 μsec/cm was always used for searching. Once an action potential was found the triggering level of the oscilloscope was adjusted to give a stable display on the oscilloscope with as little loss as possible of the positive phase of the action potentials. The orientation of the "action potential generator" could be ascertained in the following way. The position of the needle was adjusted for maximum amplitude on electrode 1, the center one in the row of three (Fig. 1). Then the electrode in the long

row showing maximum amplitude was found by switching in one electrode after the other. If the maximum was found elsewhere than at the center, then the fiber passed obliquely. In this case the fiber orientation was noted and the needle re-inserted in the corrected direction. Once the active fiber was positioned over electrodes 1 and 8, a further check was made by moving the needle slightly to-and-fro and watching the action potentials from electrodes 1 and 8. When the needle was correctly positioned the two action potentials were equal in every phase of the movement and attained maximum amplitude simultaneously. This was a very sensitive test making it possible to recognize a misalignment of less than 3°. Deviations less than 10° could be corrected by gentle adjustments *in situ*. When the long electrode row was perpendicular to the fiber, the correct position as to the rotation of the needle was ascertained by making the action potentials from electrodes 1 and 8 equal. They should then both be of maximum amplitude.

The direction of propagation could be determined by noting which of the action potentials from electrodes 1 and 8 was leading and which was trailing. Thus the innervation zone could be delineated (see page 32).

It must be emphasized that continuous readjustments of the needle position usually had to be made. It was, therefore, not possible to use any external fixation device for the multielectrode. It proved to be best to hold the multielectrode by hand, sometimes with the cable suspended so that no manual force was necessary to keep the needle in position.

For monitoring the electrode position a bipolar recording was found to be a most sensitive aid. Making the bipolarily recorded action potential of minimum size, if possible zero, meant that the muscle fiber passed strictly between the two electrodes in the bipolar pair. The screen was photographed until it was ascertained that at least 3–5 of the photographs had been made with optimum position, as judged from the bipolar recording.

The action potential nomenclature used in this investigation is given in Fig. 7.

Temperature measurements

A constantan–manganin thermo-element was used. One junction was enclosed in an injection needle, 0.5 mm in outer diameter, near the tip and the other junction was placed in an oil bath, thermostated at 35.00°C $\pm$ 0.05°C. The thermal e.m.f. was measured by a D.C. microvoltmeter (Philips GM 6020, Einthoven, Holland). The accuracy of the method was estimated to be better than 0.1°C.

Stimulation

Chemical stimulation

In order to be able to inject a solution for chemical activation of muscle fibers into a predetermined position in the muscle, a special combined injection cannula and electrode was constructed. In an opening at the side of a needle (diameter 0.6 mm) two 50 μ platinum electrodes were mounted at either side of the mouth of a glass capillary with inner diameter 150 μ (Fig. 6). The solution was injected by a motor driven syringe, connected to the needle via a long polyethylene tube, delivering 7.6 μl/sec of the stimulating solution. The electrodes could be connected either to the output transformer of the stimulator or, for recording, to an amplifier. By electrical stimulation this needle device could be positioned on the same fibers as the multielectrode, some centimeters away. After the injection of the stimulating solution the induced activity could be monitored by recording from the electrodes.

A 0.1 *M* sodium citrate solution was used for stimulation, advantage being taken of its calcium binding property. Sodium citrate is readily available in sterile solution. Chelating calcium by Versene (EDTA-disodium salt) did not offer any advantage over the citrate solution.

Electrical stimulation

A double pulse stimulator, delivering rectangular pulses of variable width, frequency, amplitude and mutual time difference was used. The output circuit was a doubly shielded transformer with the secondary shield free from ground and connected to the shaft of the stimulation electrodes, as described by Buchthal *et al.* (1955 *a*) in order to lessen stimulation artefact. A pulse-width of 130 μsec was usually used.

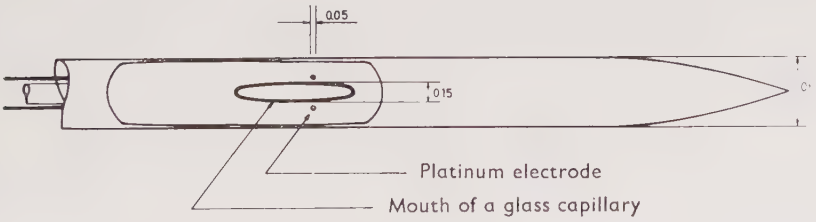

Fig. 6. *The "injection electrode".* A schematic drawing showing the two platinum electrodes on either side of the mouth of the glass capillary mounted in an opening at the side of the needle. The whole device is ground flush with the side of the needle.

Electrical properties of the recording apparatus

Test equipment

A low frequency oscillator (Krohn Hite Model 420–A, Cambridge, Mass., U.S.A.) was used, giving sine- and square waves of frequency 0.4–50,000 cps. The signals were attenuated to a voltage variable between 0 and 150 mV. The output impedance of the attenuator was 100 ohms.

The following procedure was used for feeding the signal through the multielectrodes. The needle multielectrode was inserted through the walls of a rubber cup of volume 0.1 ml (the cap of an injection bottle) so that the electrodes were exposed at the inside. The exposed part of the grounded needle stem was insulated by wax that, if necessary, could also be applied to some of the electrode surfaces. The cup was filled with normal saline. The signals were fed to a platinum needle inserted into the cup in such a way that about 10 mm^2 of the needle was exposed at the inside, which is about 10,000 times the electrode area. Resistors and capacitors that were interconnected between the platinum needle and the signal generator were soldered directly to the platinum needle. If necessary, to prevent mains interference, the assembly was wrapped in aluminium foil.

The measurements were made on the Tectronix type 502 oscilloscope.

For resistance and capacitance measurements an ESI Mod 250 DA Impedance Bridge (Electro Scientific Industries, Portland, Oregon, U.S.A.) was used.

Amplifiers and oscilloscope

The input impedance was measured by feeding a sine wave to the input cable of the cathode followers via a resistor. The voltage drop measured on the oscilloscope at 50 cps with a resistor of 100 megohm was used for calculating the input resistance, which was found to be 250 megohm. By increasing the frequency until the voltage drop with a 2 megohm resistor was 3 db, the input capacity of the input cable and the input circuits was found to be 10–15 $\mu\mu$F.

The interlead capacitance was determined by feeding an A.C. signal to one lead of the input cable via a 0.1 megohm resistor and measuring on the oscilloscope the cross-talked signal to the other leads that were at the multielectrode end of the input cable and grounded by 0.1 megohm resistances. The interlead capacity was found to be 3–5 $\mu\mu$F.

The frequency response was measured by feeding a 10 mV signal to the input cable of the cathode followers and measuring the peak amplitude on the oscilloscope. The curve was flat between 1 and 20,000 cps and reduced 3 db at 0.5 and 40,000 cps.

The rise-time was measured by feeding a 10 mV square wave to the input cable of the cathode followers. The waveform was square without overshoot. The frequency was increased until the amplitude of the wave had decreased to 80 per cent. A half period then corresponded to the 10–90 per cent rise-time, and this was found to be 8 μsec.

The phase shift was found by feeding the input signal also to the x-amplifier of the oscilloscope and watching the Lissajous figures. Phase shift was a linear function of frequency, with a delay time of 5 μsec for all frequencies within the flat part of the frequency response curve. Thus, there was no phase distortion (see TERMAN & PETIT, 1952, p. 275).

The nonlinear distortion was estimated to be negligible for signals below 150 mV for all frequencies as concluded from the study of the Lissajous figures.

The common-mode rejection was measured as the ratio between the voltage of the in-phase signal and the out-of-phase signal that gave the same output. A 1000 cps square wave was used. Common-mode rejection for any combination of input cathode followers was about 700 times. This also served as a check of equal amplification for the different cathode followers to within 0.15 per cent.

The noise-level with short circuited input and full bandwidth was 2 μV RMS. At timebase 20–100 μsec/cm the tracewidth caused by the noise of the oscilloscope was approximately 6 μV.

The non-linearity of the sweep and the vertical amplifiers of the oscilloscope was less than could be measured with the present methods.

The instability of the sweep, the "timebase jitter", i.e. the blurring of the oscilloscopic display due to different positions of consecutive sweeps when displaying a recurrent waveform, was not detectable.

The accuracy of measurement on the enlarged photograph of the screen was estimated to be ± 1 mm. For an action potential of amplitude 60 mm on the enlarged photograph this added ± 2 per cent to the uncertainty in the amplitude measurements.

The multielectrodes

The interelectrode and electrode to ground insulation resistance was more than 500 megohm.

The interelectrode capacity for all possible electrode combinations was 8.1–10.1 $\mu\mu$F.

The electrode to ground capacity measured between each electrode and the needle was 14–16 $\mu\mu$F.

The electrode impedance. The electrode impedance is both frequency and voltage dependent (Buchthal *et al.,* 1954 *a,* Burkhardt, 1957), and proved to be very difficult to measure. As the values obtained so far are uncertain, they have been omitted.

The noise level of the electrolytically treated electrodes was measured with the saline bath grounded, and was found to be 10 μV RMS, corresponding to a trace width at the oscilloscope of roughly 30 μV when using timebase 20–100 μsec/cm. The noise had the gross appearance of, and sounded like, "white noise", i.e. had the frequency components approximately evenly distributed within the passband.

Transmission characteristics of the multielectrode plus the amplifiers and the oscilloscope

The measurements were made as above. The method for measuring the frequency response and the common-mode rejection is essentially the same as described by Buchthal *et al.* (1954 *a*).

The frequency response curve was flat between 2 and 20,000 cps and reduced 3 db at 1 and 35,000 cps. The values were the same for signals between 50 μV and 150 mV.

The rise-time, 10–90 per cent, was 12 μsec. There was no overshoot in the recorded square wave.

The phaseshift. There was no appreciable phase distortion within the flat part of the frequency response curve.

Common-mode rejection was found always to be more than 200 times, and usually in the order of 500 times, for all frequencies within the passband.

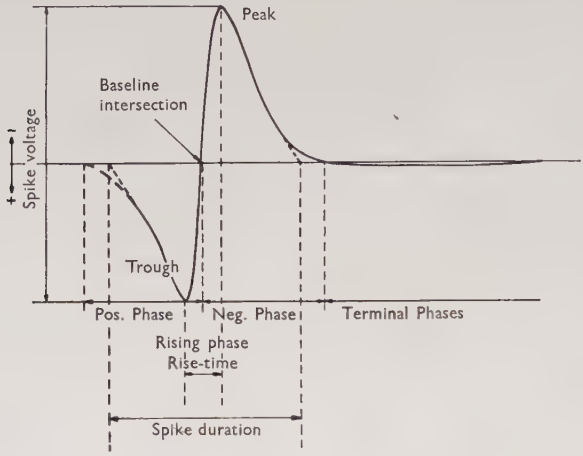

FIG. 7. *Action potential nomenclature.* "Spike" denotes the fast positive–negative phases of a clean, smooth action potential of the type shown. It is only assigned to the action potential shape, and is used irrespective of its being generated by one or several fibers. This definition is, in principal, in accordance with that used by BUCHTHAL *et al.* (1957 *a*).

As is conventional in neurophysiological work, a downward deflection on the oscilloscope indicates a positive signal. *Trough* and *peak* denote the points of maximum voltage for the positive and negative phases, respectively. Measurements of time interval between two spikes were always made between the baseline intersections.

CHAPTER 2

ACTION POTENTIALS DURING CHEMICAL ACTIVATION

THE SINGLE MUSCLE FIBER CRITERIA

Since the needle multielectrode was inserted blindly in the muscle, the conclusions concerning the action potential generator must be deduced entirely from the recordings. The 3 + 11 multielectrode was constructed to make possible recordings from the same generator at several sites of well-known mutual relation. Experimental data as to what is to be expected from the recordings of single muscle fibers *in man* are, however, lacking and therefore a method was sought to obtain a discrete fiber activity in the muscle. Electrical stimulation by needle electrodes was tried, but according to BUCHTHAL, GULD & ROSENFALCK (1955 *a*) the electrically elicited action potentials are generated by small groups of fibers and therefore this method was discontinued. Diphasic fibrillation potentials, found in denervated muscle, were also investigated, but as there is a controversy as to whether these potentials originate from single muscle fibers (DENNY-BROWN & PENNYBACKER, 1938) or from small groups of fibers (ROSENFALCK & BUCHTHAL, 1962), this method was also abandoned. Furthermore, the muscle fibers in denervated muscle are known to have electrical properties different from normal muscle fibers (e.g. NICHOLLS, 1956).

In order to elicit a muscle fiber activity in normal muscle a method of chemical activation has been developed. It is an old observation, described by KÜHNE (1859), and others before him, and correctly interpreted by RINGER (1886), that a muscle immersed in calcium-free solution becomes spontaneously active.

There is now a vast literature on the mechanisms underlying this spontaneous activity. Extensive reviews of this subject have been presented by BRINK, BRONK & LARRABEE (1946), BRINK (1954), SHANES (1958). In short, the calcium ion stabilizes the cell membrane. A lack of calcium makes the cell membrane "leaky" and the resting potential unstable. When a certain trigger value of the membrane potential is reached, that also seems to be dependent on the calcium level, a complete depolarisation process is started.

The frequency of discharge is dependent upon the amount of calcium removed.

In the method worked out an artificial "pacemaker" was created in the muscle fibers by deprivation of calcium brought about by local injection of a minute quantity of a sodium citrate solution. The local application of a citrate solution to a frog muscle preparation has been described by ADRIAN & GELFAN (1933) and to the frog nerve by BRINK *et al.* (1946), but has not been previously used as a method for producing fiber activity in the mammalian muscle *in situ*.

It will be shown, that by this method it is possible to induce an asynchronous activity in individual muscle fibers and, consequently, the method can be used to define the criteria to be fulfilled by single muscle fiber action potentials.

Procedures

It was found to be difficult simply to inject the stimulating solution using an ordinary syringe and then try to find the stimulated fibers with the recording electrode. Therefore it was convenient to position the injection cannula and the recording electrode on the same small group of fibers, and this was done by electrical stimulation.

The short head of the brachial biceps muscle was chosen for these experiments, as the fiber direction is known and the innervation zone is principally confined to the proximal and the central part of the muscle (BUCHTHAL *et al.*, 1955 *a*; CHRISTENSEN, 1959). By making the injection distally in the muscle, the risk of activating terminal nerve fibers ought to be small.

To be able to detect action potentials reflexly evoked by activation of sensory structures and those caused by voluntary activation, the multielectrode was inserted distally to the innervation zone, which was checked by noting the direction of propagation of the voluntary action potentials picked up by the multielectrode (see page 26). It was ensured that the multielectrode was entirely outside the innervation zone by observing about 30 different voluntary action potentials at different sites in the same cross section of the muscle.

The "injection electrode" (Fig. 6) was then inserted some centimeters more distally in the muscle. In order to place the stimulating and recording electrodes on the same fibers, the procedures of BUCHTHAL *et al.* (1955 *a*) were used. The potentials evoked were sought with a high stimulating voltage. By decreasing the voltage and continously adjusting the position of the two electrodes it could thus finally be ascertained that the two electrodes really were located near the same fibers.

In this position the electrodes previously used for stimulation were coupled to one amplifier channel for monitoring the activity, and a few microliters of the 0.1 *M* sodium citrate solution were injected (see page 27).

There could be a delay of up to a few minutes before the spontaneous activity appeared. Then a few spike sources within the uptake area started firing. Their behaviour was, however, capricious. They could suddenly cease or start to dis-

charge violently without any obvious reason. The initial positioning of the injection cannula and the multielectrode could only be approximate, and once the chemical activity had started, the multielectrode had to be re-positioned on one of the active spike sources.

Action potential recording

The general procedures given on page 24 were followed. The 3 + 11 multielectrode was used. The following program could rarely be completed during the short time available due to the capricious behaviour of the action potentials. Therefore it was necessary to concentrate on a certain part of the program for each recording, so that a complete picture was obtained from the whole sample of recordings.

Recording program

1. Ascertaining the needle position so that electrodes 1 and 8 were correctly aligned with the spike source.
2. Checking the direction of propagation of the action potentials so as to be sure that the impulse had been initiated near the site of injection.
3. Recording the action potentials from electrodes 1 and 8, both with the needle in optimum position and when moving the needle to-and-fro.
4. Comparisons of the shape of consecutive action potentials, both directly on the oscilloscope and indirectly from photographs with at least five superimposed action potentials.
5. Recording from all the electrodes in the long electrode row, with electrodes 1 and 8 aligned with the spike source.
6. Simultaneous study of the bipolar recordings 9–8 and 9–10 while moving the needle to-and-fro. For the reasons for using these electrode combinations see page 18.

Material

Fourteen experiments have been performed in eight young adults without gross signs of neuromuscular disorders. Recordings, based on the above program, have been made from about 500 different spike sources but, as mentioned previously, only a part of the program has been performed on each.

Results and discussion

The type of activity elicited by chemical stimulation

Results

The injection of a minute quantity, 10–20 μl, of the stimulating solution usually elicited a sparse activity by only a few of the spike sources within the uptake area of the electrode. The discharge rate was of the order of

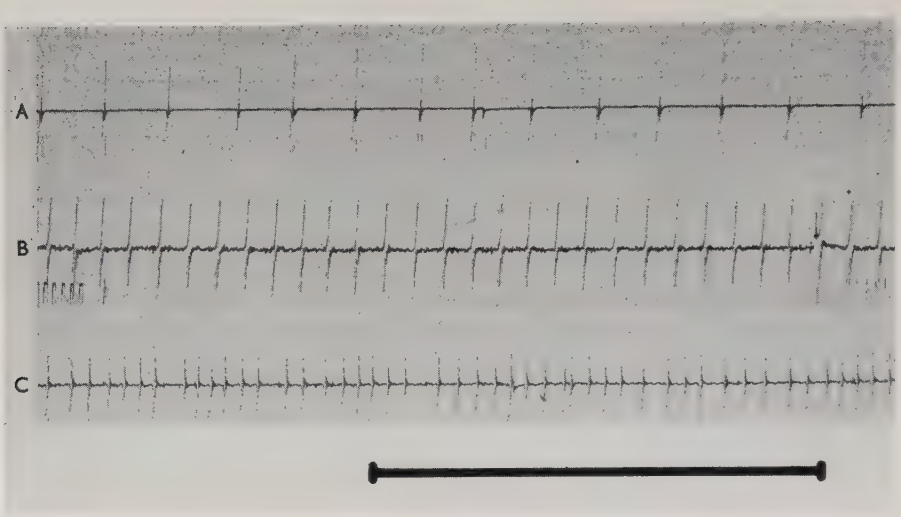

FIG. 8. *Ink-recordings of chemically elicited activity from single muscle fibers at different repetition rates.* Calibration: 1 sec.

1–5/sec. The activity was like that of "fibrillation" at denervation (Fig. 8). Injecting more citrate solution increased the activity so that more spike sources became active, and the discharge rate sometimes increased to give a chaotic interference pattern. After the injection of larger amounts of citrate, a grouping of the spikes occasionally appeared, like "fasciculations", so that 2–4 potentials discharged together (Fig. 9 *A*). However, there was a considerable variability of an order of magnitude of milliseconds in the mutual time interval, so that even the order of the components could vary from discharge to discharge. Often one of the components would miss a discharge. The variability was usually so gross as to be detectable on the ink-recorder with a paper speed 50 mm/sec (Fig. 9 *A, B, C*).

Sometimes there were repetitive discharges in bursts of 2–5 potentials, all from the same generator (Fig. 9 *D*), as commonly seen in tetany. The mutual time interval could vary between 3.5 and 8.0 msec, but was usually rather constant between the components and from burst to burst. It cannot be settled with certainty if this type of activity was dependent upon the amount of citrate injected or the site of injection.

Another type of activity were intense bursts with repetition rates of the action potentials up to 200 per second and a duration of 0.5–3 seconds (Fig. 9 *E*). This activity seemed to have been generated by one single source. The burst could start and stop either suddenly or gradually, like the "dive-bomber" phenomenon encountered in myotonic dystrophy. It seemed as if

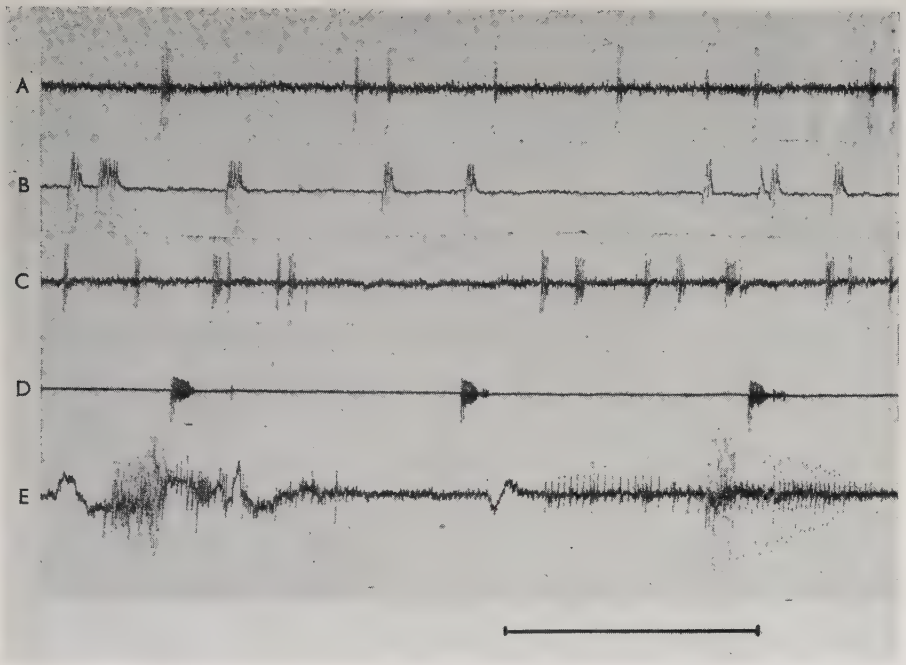

FIG. 9. *Ink-recordings of action potential groupings (A, B and C), repetitive discharges (D) and the "dive-bomber phenomenon" during chemical activation (E).* See page 34. Calibration: *A, C, D* and *E* 1 sec, *B* 0.5 sec.

these last-mentioned types of activity could only be elicited from certain rare sites in the muscle.

All the different types of activity could usually be started or intensified by the mechanical stimulation that was obtained by moving the injection needle or tapping with a finger.

Discussion

It seems most likely to assume that the regular and quiet "fibrillatory" activity most commonly encountered actually derived from ordinary (extrafusal) muscle fibers. It is, however, essential to know if the activity really was *single* fiber activity or if more than one fiber was involved in generation. The present finding of "grouping" of the action potentials after the injection of greater amounts of citrate is of special interest in this respect. Due to the gross variability in the mutual time intervals and because the citrate was injected in a part of the muscle where motor end-plates are sparse, it does not seem likely that the activity could have been evoked by stimulation of intramuscular motor nerve fibers. However, partial synchro-

nization of muscle fibers in calcium deficient solutions was first reported in the frog muscle preparation by ADRIAN & GELFAN (1933). They found the phenomenon also after curarization, so excluding nerve stimulation as a causative factor. The conditions under which synchronization occurred in those experiments seem to resemble those in the present experiments. They found that the synchronization waves "only occur at high frequencies: the maximum has varied from 90–120 a sec, and the minimum has never been less than 60. When the frequency has fallen to this value the discharge breaks up into the usual rapid, irregular type, though an occasional large wave may appear from time to time." The authors state that the essential conditions for such an occurrence seem to be, "(*a*) an intense and fairly uniform activity in several fibres, and (*b*) the existence of damaged or permeable regions at neighbouring points in them. These would bring the interiors of the fibres into free electrical communication and give a chance to the group to behave as a single unit. An active region developing in one fibre close to the injury would tend to activate not only the neighbouring points in the same fibre but also those in the fibres next to it, and thus a rhythmic discharge in one fibre might come to dominate the rhythm of its neighbours." BÜLBRING, HOLMAN & LÜLLMANN (1956) studied the frog muscle in a calcium deficient solution under the microscope and confirmed the observations of ADRIAN & GELFAN (1933) that at moderate activity the fibers fired asynchronously. Further, they found that not all neighbouring fibers were active at the same time. Intracellular recordings showed that the degree of depolarisation necessary for "triggering" was constant within 5 mV for a certain fiber but differed from the voltage needed in the other fibers of the preparation. They also observed partial synchronization phenomena at higher degrees of activity, especially in conjunction with mechanical stimulation or after application of histamine.

KUFFLER (1944) described the activity of the muscle in calcium free solution as a non-synchronous single fiber activity but, at times, synchronized action potentials could appear, and these he interpreted as due to a mutual influence of partly depolarised *end-plate* regions.

TAMAI, ABE & GOTO (1961) described a synchronization phenomenon due to direct interaction between adjacent fibers in a frog muscle preparation in a calcium-free solution; the synchronization was, however, no obligate phenomenon, but could happen now and then at the height of the excitability increase.

Both in the present and in previous investigations it has thus been found that an action potential grouping with great temporal dispersion and with *loose* binding of the component action potentials may exist. It must, however, be emphasized that in all the previous experiments that were described

above, the partial synchronization phenomenon was observed with *the whole* muscle immersed in calcium-deficient solution. In the present experiments a *small part* of the muscle fiber was made deficient in calcium, and this was at least 20 mm away from the recording site. The partial synchronization seems to appear only after application of larger amounts of citrate. Thus it is most unlikely that synchrony without *any* temporal dispersion and with *fixed* binding of the components—i.e. equal to within a few microseconds from discharge to discharge—should exist at the low degree of activity obtained after the injection of a minute quantity of citrate. The conditions for mutual interaction ought then to be considerably less. *The action potentials of the fibrillatory type at low discharge rate seem thus to be generated by single muscle fibers.*

The high frequency bursts of activity and the "divebomber" phenomenon were strikingly similar to the type of activity that has been attributed to intrafusal muscle fibers by BONSETT & ABREU (1963). These authors were able to record this type of activity in advanced cases of muscular dystrophy, where the muscle spindles are known to survive until late in the progress of the disease, and could obtain histological evidence for their recording using the "electromyotome" (cf. page 15). The intrafusal muscle fibers are also known to become spontaneously active upon calcium deprivation (e.g. BRUGGENCATE & SCHULTE, 1963). The present finding that mechanical stimuli could initiate the activity and that it could be elicited only in certain positions of the injection cannula further supports the above interpretation. The question needs, however, further study before definite conclusions can be drawn.

The single fiber action potential recordings

Results

The action potentials recorded at slight activity, of the type considered to have been generated by single muscle fibers, were smooth biphasic spike potentials (Fig. 10) of a shape similar to those recorded from the isolated frog muscle fibers by HÅKANSSON (1956, 1957 *a*). A third positive phase of low amplitude and long duration was sometimes seen. Action potentials of amplitude up to 12 mV were recorded. At least 10 action potentials were about 10 mV in amplitude. The spike duration varied between 500 and 1000 μsec and the rise-time was 80–150 μsec. With increasing recording distance the voltage decreased and the rise-time increased, but the action potential shape was always clean and smooth, without irregularities.

The action potentials from electrodes 1 and 8 could be made equal in shape and voltage by rotation adjustments of the needle (after correction for the cross-talk distortion pages 17 and 75). When moving the needle to-

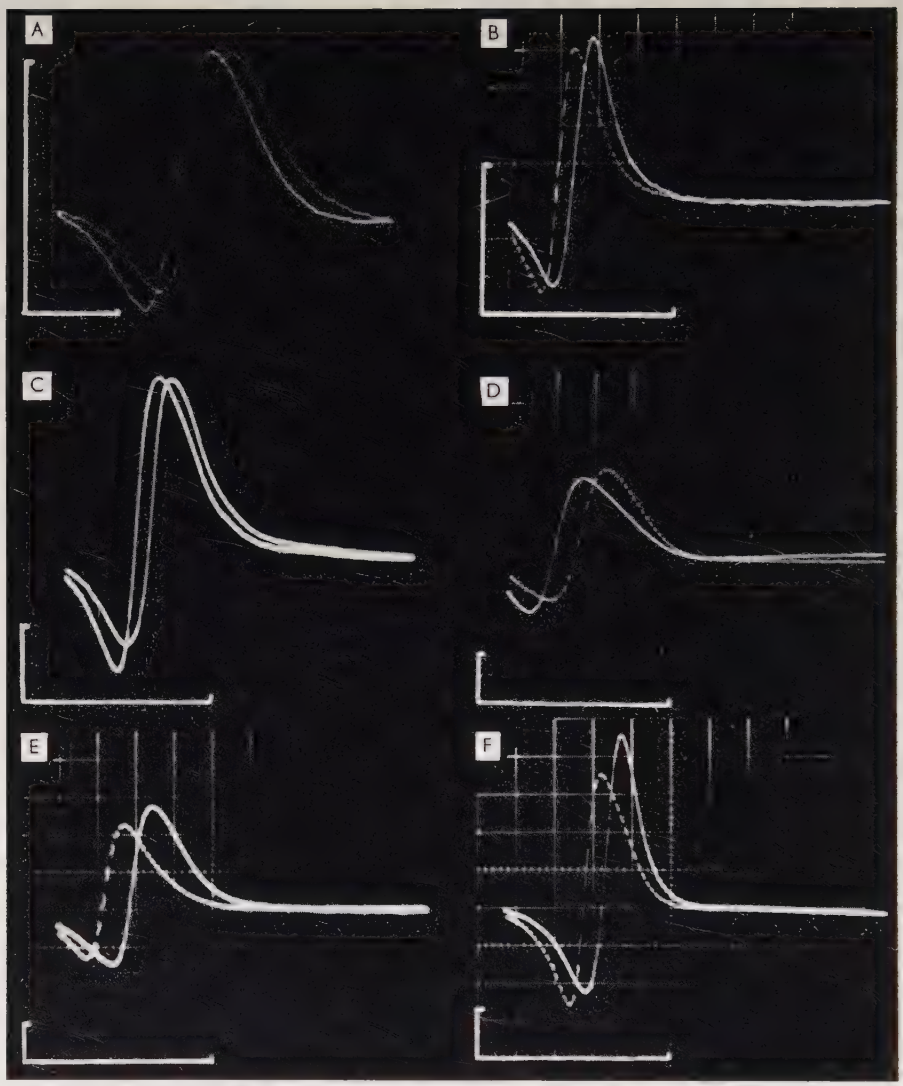

FIG. 10. *Examples of chemically elicited action potentials.* Single sweep recordings from electrodes 1 and 8 of the 3 + 11 multielectrode. Calibration: 2 mV and 500 μsec.

and-fro the action potentials were always equal and attained maximum voltages for the same needle position, provided the electrodes 1 and 8 were correctly aligned with the fiber.

From any electrode, which was kept in constant position to the fiber, the consecutive action potentials were of identical shape (Fig. 11) if the discharge

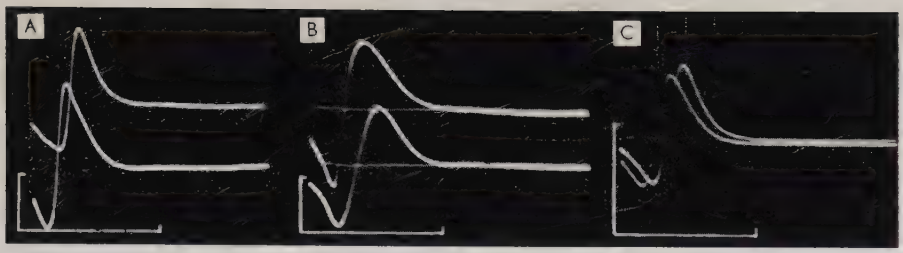

FIG. 11. *Superimpositions of consecutive action potentials obtained during chemical activation.* Recordings from electrodes 1 and 8 of the 3 + 11 multielectrode. Five discharges are superimposed in Fig. *A*, eight in Fig. *B* and ten in Fig. *C*. Calibration: 2 mV and 500 μsec.

rate was low. In the repetitive bursts of activity the amplitude was sometimes continuously decreasing within each burst.

It was very difficult to obtain suitable recordings from the different electrodes in the long row as this procedure was rather time consuming. Therefore, it has not been possible to obtain exact quantitative data, but the following observations were made: There was a steep voltage decrement with increasing distance (cf. Fig. 34, page 73). Action potentials recorded at the same distance from either side of the fiber were of equal shape and amplitude and were simultaneous. The baseline-intersection was simultaneous for the action potentials from all electrodes.

The bipolar recordings from electrodes 9–8, on one channel, and 9–10, on the other, could usually be made equal in shape and voltage and simultaneous, and this happened for just that needle position when both were of the maximal attainable voltage. The maximum voltage was usually $^1/_2$–$^1/_3$ of the voltage of the monopolar action potential from electrode 9, which was also of maximum voltage in the same position. By adjusting the needle position, either of the bipolar recordings could be made successively smaller until appearing as a nearly straight line. Moving the needle further in the same direction made the action potentials increase again but in a reversed form. The appearence of a bipolar recording obtained at voluntary contraction is shown in Fig. 28, page 62.

Discussion of the single muscle fiber criteria

Our knowledge of the single muscle fiber action potentials, led off at different distances from the fiber, is mainly due to the experiments on isolated frog muscle fibers of HÅKANSSON (1956, 1957 *a, b*). There are considerable quantitative differences between the human and the frog recordings. No direct comparisons can be made, because in the present investigation the

exact recording distances and the fiber diameters were not known. However, it can be concluded from the data of Håkansson that, if using the 3+11 multielectrode for the isolated frog muscle fibers, essentially the same types of recordings probably should have been obtained as in the present investigation. Thus the recordings from the isolated frog fibers ought to fulfil the present single fiber criteria.

In deciding upon the single muscle fiber criteria it has seemed wise not to use any quantitative information but only the general shape of the action potentials under the different conditions.

The aim of the recordings of the chemically elicited activity has been to decide the single fiber criteria. The principal discussion of the action potentials will be presented in Chapters 3 and 4 using the data from the recordings at voluntary contraction, as the information thus obtained is far more detailed and the number of recordings much larger.

The single muscle fiber criteria

The recordings from a single muscle fiber aligned with electrodes 1 and 8 in the 3+11 multielectrode (Fig. 1) fulfil the following criteria.

1. The action potential shape recorded from any of the electrodes is a clean and smooth biphasic spike (Fig. 10).

2. The action potentials from electrodes 1 and 8 are of equal shape when the needle position is adjusted to make both potentials of maximum voltage (Fig. 10). They then also equal each other in all needle positions obtained by to-and-fro movements.

3. Consecutive action potentials are identical in shape (Fig. 11).

4. The action potentials from the different electrodes in the long row have simultaneous baseline intersections and a steep and symmetrical voltage decrement with increasing distance (see Fig. 34).

5. The bipolar recordings 9–8 and 9–10 can be made equal and simultaneous, and this occurs in that needle position where they are both of maximum voltage. The monopolar action potential from electrode 9 is then also maximal. By to-and-fro adjustment of the needle position, either of the bipolar action potentials can be made successively smaller down to zero. The baseline intersections for the action potentials obtained at all needle positions are simultaneous (see Fig. 28).

CHAPTER 3

ACTION POTENTIALS DURING VOLUNTARY CONTRACTION

The conditions for recording a single muscle fiber action potential in the voluntarily activated muscle are that the fiber is close to the electrode and thus gives rise to an action potential of high amplitude, and that those other fibers of the motor unit that have coincident action potentials are remote enough from the electrode to make their contribution small. Such a potential must fulfil the single muscle fiber criteria (page 40) even if it is not "single" in the strict sense of the word. However, are there any conditions under which it is possible that the compound action potentials from two or more adjacent fibers, that all give appreciable contributions, might fulfil the single muscle fiber criteria? The principal aim of this chapter is to answer this question. This has been done by studying *obviously composite* potentials with regard to the criteria in order to gain information concerning what is to be expected from composite potentials of *apparently simple shape*. Some quantitative aspects of this question have been studied by means of "synthetic" composite potentials obtained by a method of adding simple action potentials of known shape, amplitude and mutual time interval.

The question of the minimum detectable degree of interference from remote fibers on a certain single muscle fiber action potential has also been treated.

No systematic study of single muscle fiber action potentials has been found in the literature. Usually the possibility of such recordings has not even been taken into account. Only BUCHTHAL *et al.* (1957 *a*) in discussing the spike of the motor unit potential say that ". . . motor unit spikes with low maximum amplitudes derive from sources with a smaller radius than the sources of high amplitude spikes. As to the minimum size of the potential source, the estimate obtained from volume conduction does not exclude that the source may consist of a single fibre only". ADAMS *et al.* (1962, page 117) have concluded that all attempts to record single fiber action potentials have failed (cited here on page 6).

Material and procedures

Material

Most recordings have been made in the biceps and the extensor digitorum communis muscles of the arm, mainly because these muscles are very easily controlled by the subject. After the final settlement of the single fiber criteria about three thousand recordings fulfilling the single muscle fiber criteria have been studied as well as a similar number of recordings where the generator has been two or more muscle fibers. This has been made in 105 experiments on 28 subjects, mostly young adults, but including two children $4\,^1/_2$ and $6\,^1/_2$ years old. The oldest subject was 58 years. Seven of the subjects were women. None had any signs of neuromuscular disorder.

Procedures

With the subject prone and the arm comfortably positioned, the muscle was slightly contracted against a resistance. No attempts were made to make the contraction strictly isometrical and the force was not measured. The slightest possible effort was used to facilitate maintainance of the contraction for a long period and to lessen the effects of fatigue. The usual discharge rate of the motor unit was 5–10 per second. The room temperature in the earlier experiments was 30–34°C and in the later experiments 22–24°C, and, at least in the later experiments, fairly constant during the course of an experiment (2–3 hours).

In most of the experiments intramuscular temperature measurements have been performed (see page 26). The needle thermo-electrode was in constant position during the course of an experiment because it could not, for practical reasons, be brought near to the multielectrode each time this was repositioned. As there proved to be considerable temperature gradients within the muscle, as also shown by Bazett & McGlone (1927) and Buchthal, Høncke & Lindhard (1944), the temperature readings could not be considered as a measure of the temperature of the muscle fibers in the region from which the recording was made. Therefore, the temperature readings in each individual recording are not given. They generally fell between 34° and 36°C.

The multielectrode was usually inserted about halfway between the end-plate zone and the tendon, according to the procedures described on page 24 ff. A perpendicular position of the electrode row in relation to the muscle fiber was always sought.

Simulation of composite potentials

The shape of a composite potential composed of two *simultaneous* single fiber potentials is as clean and smooth as each of the components. Only if there is a sufficiently large time interval between the components will an irregularity be seen in the shape. What is the minimum detectable time interval? To answer this and similar questions a method was developed where action potentials of known shape, amplitude and mutual time interval were electronically added to give a "synthetic" composite potential.

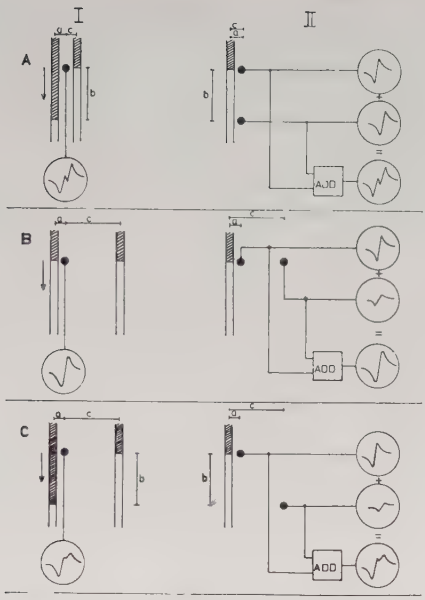

FIG. 12. *Simulation of composite potentials.* The composite potentials obtained by recording the action potentials from *two* identical fibers with *one* electrode (column I) equal those obtained by adding the action potentials from *one* of the fibers recorded with *two* electrodes (column II). *a* and *c* represent the fiber–electrode distances, *b* represents the distance between the depolarisation fronts of the two fibers (column I) and the electrode separation (column II). *A*, equidistant fibers with a time interval between their action potentials. *B*, fibers at different distances from the electrode with simultaneous action potentials. *C*, fibers at different distances from the electrode with a time interval between their action potentials.

Theoretical background. In a homogeneous volume conductor the current distribution due to an electromotive force (e.m.f.) is independent of the presence of any other e.m.f. The voltage in a point caused by several e.m.f. will then equal the algebraic sum of the voltage that each e.m.f. should have caused in the absence of the others (HELMHOLTZ, 1853). This will also be the case if the volume conductor is inhomogeneous or anisotropic but not in the presence of any non-linear elements, i.e. rectifiers or voltage dependent impedances. BURKHARDT (1957) remarks that "bei extracellularen Untersuchungen können die Nichtlinearitäten durch unphysiologisch hohe Spannungen zwar nachgewiesen werden, sie spielen aber normalerweise kaum eine Rolle". Therefore it can be assumed that, the results obtained by adding action potentials *outside* the muscle are equal to those obtained when the action potentials are added *within* the muscle. BUCHTHAL (1960, 1961) has made analyses of compound potentials based upon the same assumption.

Addition of action potentials of different voltage, shape and mutual time interval can be made as a purely geometrical construction. However, this is

tedious and can definitely not be used for determining the minimum degree of irregularity that will be detected in an action potential waveform. The determinations must be made on the directly observed oscilloscope and on the photographs of the oscilloscopic display in order to include all possible factors. Therefore a method was developed for simulation of composite action potentials at the moment of recording. The principles are sketched in Fig. 12 and can be briefly summarized in the following way: The composite potential from *two fibers,* recorded with *one electrode,* may be simulated by adding the action potentials recorded with *two electrodes* from *one fiber.* The interelectrode distance measured perpendicularily to the fiber axis then corresponds to the distance between the simulated and the real fiber. The interelectrode distance along the axis of the real fiber corresponds to the distance between the depolarisation fronts of the propagation processes for the real and the simulated fiber.

Procedures. Both the $3+11$ and 3×4 multielectrodes (Figs. 1 and 2, pages 17 and 19) were used, the latter mainly when simulating temporal dispersion between the spikes. The following refers to the 3×4 multielectrode.

With electrodes 1 and 9 aligned with a fiber the condition in Fig. 12 *A* could be obtained by adding the action potentials from electrodes 1 and 9, the condition in Fig. 12 *B* by adding those from 1 and 4 and the condition in Fig. 12 *C* by adding those from 1 and 12. The irregular electrode array gave many different similar combinations, representing different spatial and temporal intervals. For a fiber with propagation velocity 3.5 meter/sec the following time intervals between two equal spikes could be obtained: 14, 23, 27, 29, 30, 41, 46, 52, 55, 69, 70, 73 μsec. By using fibers with different propagation velocity other time intervals, ranging from 11 to 100 μsec, were obtained.

Action potentials that fulfilled the single fiber criteria were used.

A typical simulation experiment was performed as follows:

On channel I of the oscilloscope the simple spike was displayed. On channel II the composite potential obtained by adding the output from two electrodes was shown. Different time intervals between the two components were selected in random order and it was determined at which time interval the composite character was detected (*a*) without reference to the simple potential and (*b*) when using the simple potential for comparison. The determinations were made by direct observations on the oscilloscope screen and on photographs. Ten experiments of this type were made.

The procedure for using the $3+11$ multielectrode in simulation experiments is described on page 58.

The "double spike"

One type of recording that proved to be a most useful tool in the evaluation of the single muscle fiber criteria was the "double spike" (Fig. 13), i.e. two action potentials from the same motor unit, each of them fulfilling the single fiber criteria and with a time interval sufficient to prevent interference between them.

That the two spikes belonged to the same motor unit was beyond doubt because of their coupling at different discharge rates (cf. page 9). The double spikes could not possibly have been repetitive discharges from one single fiber

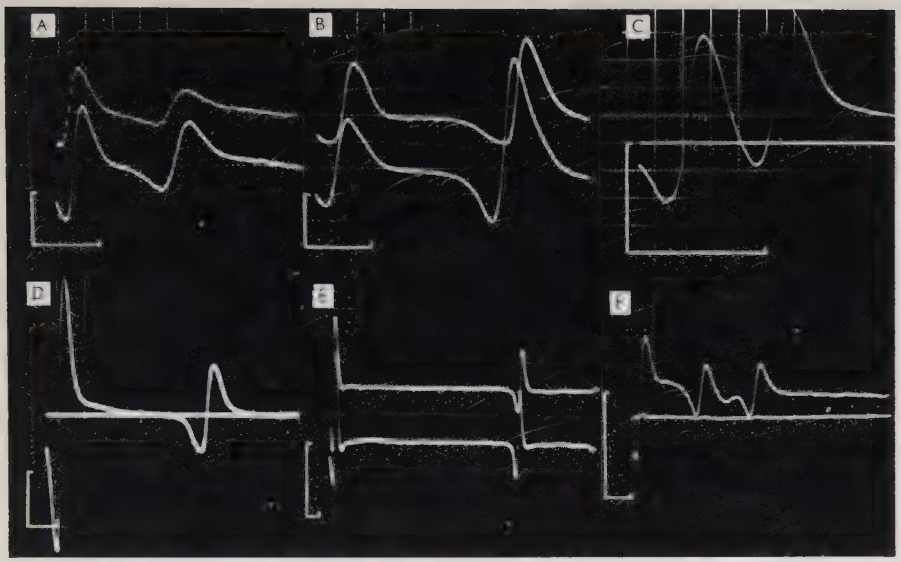

FIG. 13. *"Double spikes"* and *"triple spikes"*. See page 44. Recordings from electrodes 1 and 8 (*A, B, E*) or only 8 (*C, D, F*) of the 3+11 multielectrode. Calibration: 2 mV and 500 μsec.

because the two components usually were of maximum amplitude on different electrodes. The double spikes were recorded either from one electrode positioned in such a way that the two components were of equal voltage, or each component was recorded from its electrode of maximum amplitude on separate amplifier channels. Recording could also be made by bipolar lead-off from the two electrodes or by adding the signals from the two electrodes by means of the addition device in the amplifier. The double spike had a characteristic "hollow" sound.

Results

General remarks on the activity recorded with the multielectrodes

The special design of the multielectrode with the electrodes mounted in an opening at the side of the needle, with the small electrode area and with the insulating wall around the electrodes, made the electromyogram somewhat different to that obtained by ordinary concentric needle electrodes. The main general characteristics of the recordings will be summarized briefly.

At rest there was electrical silence, except for the occasional occurrence of "end-plate noise" (BUCHTHAL, 1961), i.e. miniature end-plate potentials when the electrode was positioned in the immediate vicinity of a neuromuscular junction. They were purely negative spikes, with amplitude up to 200 μV, mainly confined to one of the electrodes.

There was a striking absence of insertion activity, and this was probably due to the fact that the fibers which were mechanically activated by the tip of the needle were outside the uptake region of the electrodes at the side of the needle. Occasionally, however, a train of impulses, sometimes combined with a dull pain, was seen, similar to the postulated intrafusal muscle fiber activity found during chemical activation (see page 37).

The action potentials obtained during slight or moderate contraction showed a rich variety of different waveforms, the simplest being the fast, smooth spike that fulfilled the single fiber criteria. Most other potentials seemed to be composed of two or more such spikes of different voltage and mutual time interval. Up to eleven spikes could be discerned in a "polyphasic" potential. In composite potentials of low voltage (less than 0.5 mV) the individual spikes might be difficult to discern, appearing mainly as humps or other irregularities of the waveform.

The maximum potential voltage obtained with the multielectrode was considerably greater than that with a concentric electrode. Maximally, 38 mV was obtained (for a composite potential) which is nearly ten times that which has been reported in normal muscle with concentric electrodes (4.2 mV according to BUCHTHAL *et al.*, 1957 *a*).

At increasing effort the electromyographic activity successively increased up to a chaotic interference pattern. The individual action potentials were difficult to discern above a contraction strength of more than about half the maximum because too many motor units were active within the uptake area of the electrode.

Tests of the single fiber criteria on composite potentials

In all cases the testing of the criteria was made both by direct inspection of the oscilloscopic display when making the experiment and indirectly from the photographs. Judgement of the action potential shape proved to be nearly as accurate when made directly on the oscilloscope as on the photographs. All voltage and time measurements were made on the photographs.

The action potential shape

The single muscle fiber criterion: The action potential shape recorded from any of the electrodes is a clean and smooth biphasic spike (Fig. 10).

Simulation experiments

The results have been obtained from the experiments described on page 44. When two equal and simultaneous action potentials were added a composite potential of *the same shape* but of double amplitude was obtained.

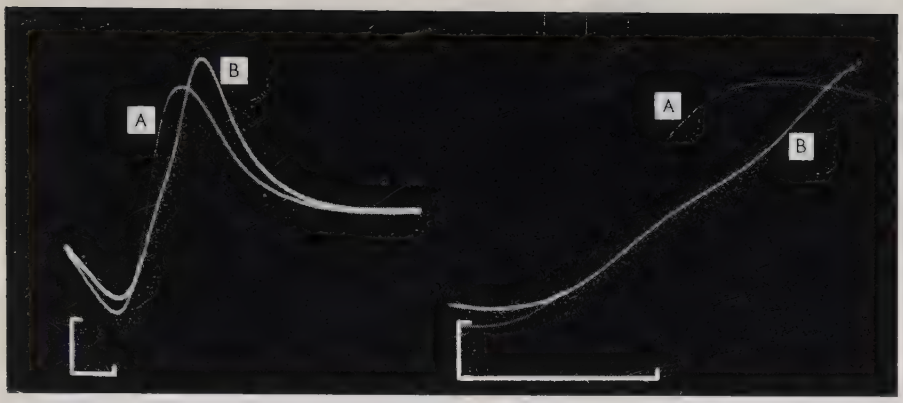

FIG. 14. *Simulated composite potentials.* Two equal spike potentials of voltage 8.6 mV and rise-time 120 μsec added without time interval (*A*) and with time interval 40 μsec (*B*). Recordings from electrodes 1+1 (*A*) and 1+8 (*B*) of the 3+11 multi-electrode. Two different sweep speeds are shown. Calibration: 4 mV and 100 μsec.

At increasing time difference between the action potentials there was a deformation of the rising phase (Fig. 14) and when the time interval exceeded the duration of the individual rising phase a notch appeared (Fig. 15). It was found that a composite action potential composed of two equal components definitely revealed its character without reference to a simultaneously visible simple one if the time dispersion between the components was more than one third of the individual rise-time (as in Fig. 14), i.e. of an order of 30–60 μsec.

If the component action potentials were not of the same amplitude the distortions in the wave form altered in character. If the trailing component was smaller, the notch was closer to the peak, if the leading component was smaller, then the notch was closer to the trough.

The action potentials from electrodes 1 and 8

The single muscle fiber criterion: The action potentials recorded from electrodes 1 and 8 are of equal shape when the needle position is adjusted to make both of maximum voltage (Fig. 10). They then equal each other also in all needle positions obtained by to-and-fro movements.

Actual composite potentials

In spite of the small distance (0.2 mm) between the two electrodes the action potentials from the central electrodes in each row were sometimes grossly unequal (Fig. 16). This occurred also when the sources of the composite potential were situated close together (30–60 μ), as judged from the sites of maximum amplitudes for the individual components. Sometimes the

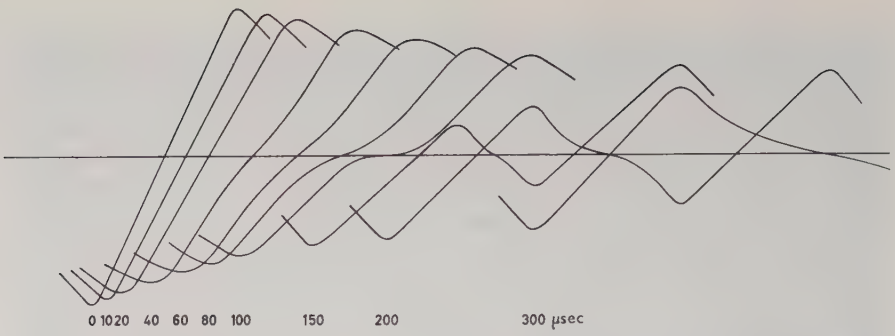

FIG. 15. *Shape of composite potentials* composed of two equal components with increasing time interval. A graphical construction. Rise-time of the component action potentials is 100 μsec. The numbers below the potentials indicate the time interval in μsec between the components. Only the first part of the composite potential is shown for the 300 μsec case.

shape on one electrode was rather smooth and clean, but on the other clearly composite, even when the two sources were shown to be at a center-to-center distance of 60 μ or less (Fig. 16 *B, D*).

Simulation experiments

Assume that the composite potentials appear clean and simple on electrode 1. Then one wants to know what is needed for a change to be apparent on electrode 8. The simulation was performed as under *b* on page 44. The minimum time interval obtained was 11 μsec and this gave a very easily recognizable difference between the two potentials. It can be concluded that even 5 μsec would be easily detected.

The only possible cause for such a difference is a different propagation velocity for the two fibers. With one fiber action potential travelling at 3.5 meter/sec the other could then have a velocity of 3.2–3.85 meter/sec without the composite character being detected. If the two action potentials were of different voltage, and the distortion of the shape thus was in the peak or trough, then the sensitivity in detection of the composite character was less.

Consecutive action potentials:

The jitter phenomenon

The single muscle fiber criterion: Consecutive action potentials are identical in shape (Fig. 11).

Actual composite potentials

The action potential shape was not identical from discharge to discharge in *any* of the 3000 composite potential recordings exceeding 0.5 mV in

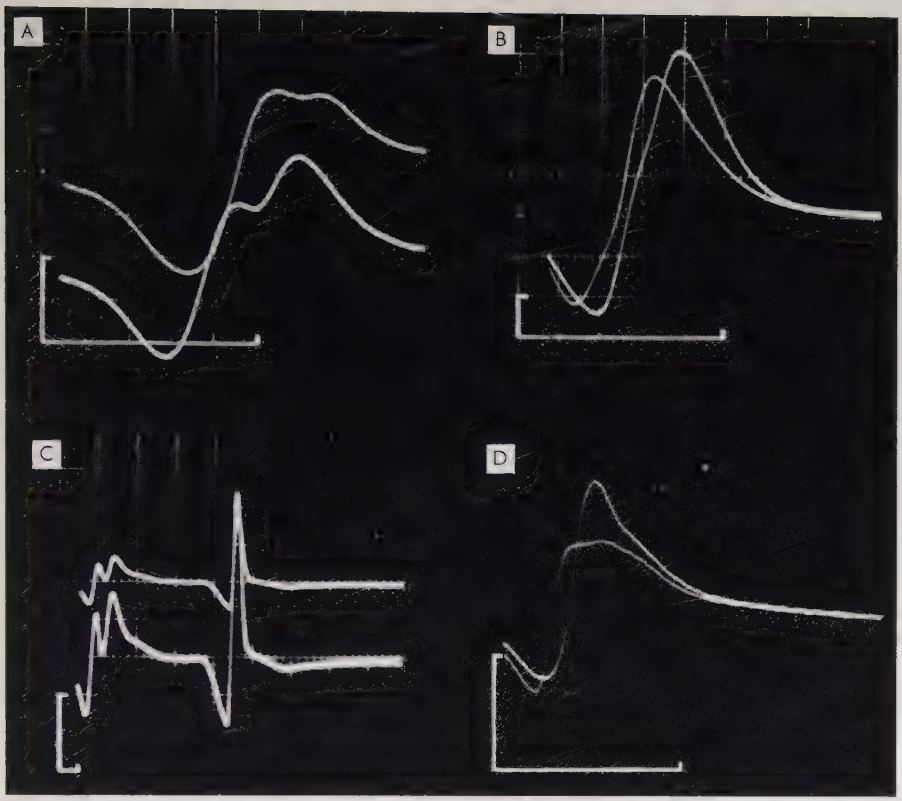

FIG. 16. *Composite potentials* differing in shape on electrodes 1 and 8 of the 3+11 multielectrode. In *A* and *B* the center-to-center distances between the individual spike sources were about 60 μ. Calibration: 2 mV and 500 μsec.

voltage made with the present method, nor in any of some 2000 such records made with earlier techniques. There was always a variation in the shape, usually confined only to a part of the potential (Fig. 17). When observed on the oscilloscope the variations were seen as an apparent movement of a part of the display in a restless, nervous manner; hence called the *"jitter phenomenon"*. ("Jitter" is adopted from the technical literature where it denotes an instability in an oscilloscopic display due to e.g. variations in the triggering level or instability in the time-base generator.)

By the study of composite potentials where two spikes were clearly discernable, preferably "double spikes" (page 44), it was found that the cause of the jitter was a *variability in the mutual time interval between the spikes* and that the individual spikes were of identical shape from discharge to discharge (Fig. 18). (A photograph of 58 superimposed consecutive spikes

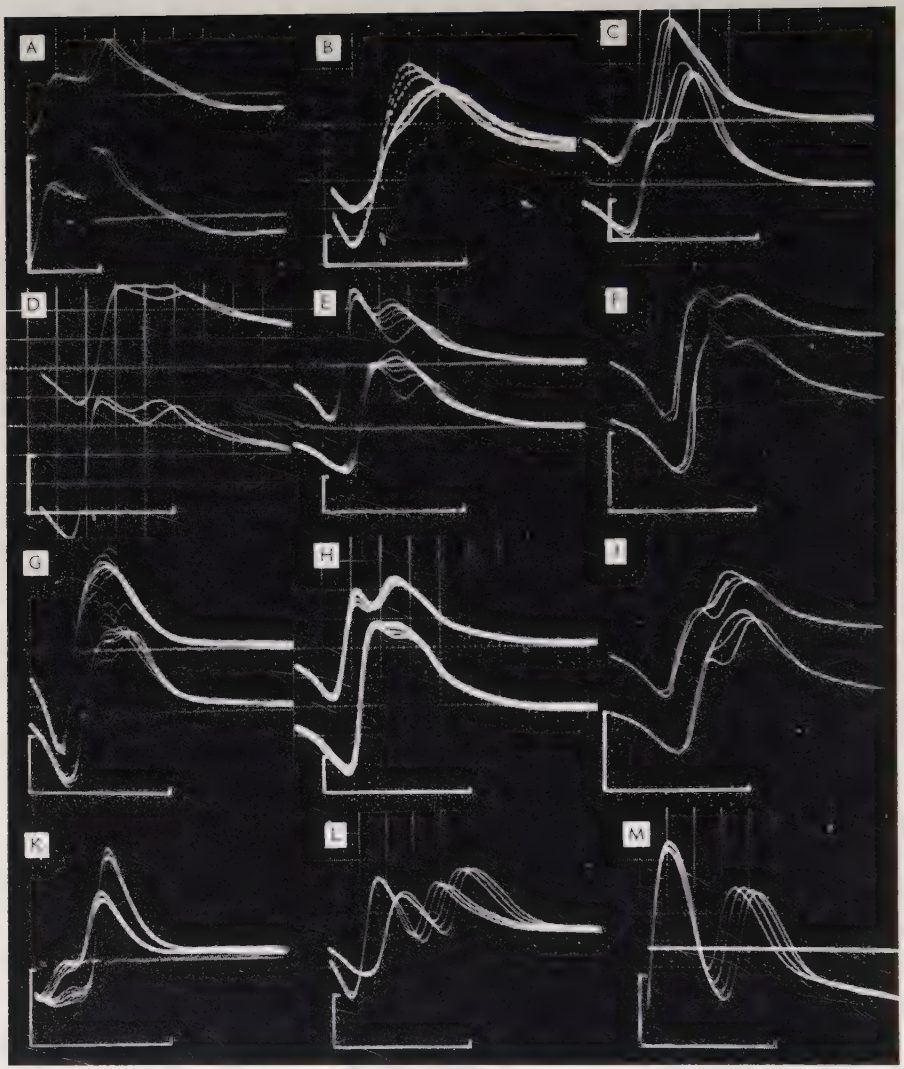

FIG. 17. *Action potential jitter.* Several consecutive discharges of composite potentials superimposed on each photograph. Electrodes 1 and 8 of the 3 + 11 multielectrode. In *A, C, J, L, M* the center-to-center distance between the individual spike sources proved to be 60 μ or less. *C, F, J* have been retouched. Calibration: 2 mV and 500 μsec.

is shown in Fig. 19.) As the sweep was triggered by the first spike, this had a locked position on the oscilloscope at all consecutive discharges, and the time interval differences were reflected as an apparent movement of only the second spike. This must not be misinterpreted as there being time variations only in the arrival time of the second spike; it is in fact the *difference*

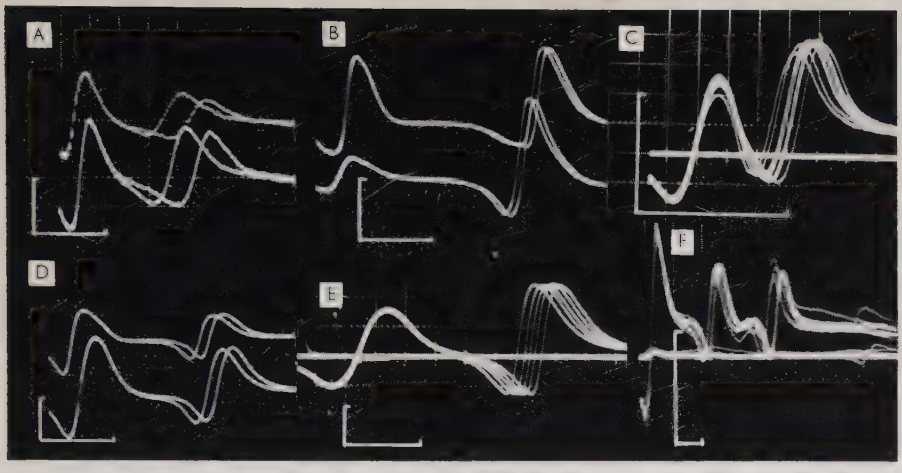

FIG. 18. *Jitter in "double spikes".* Superimposed consecutive discharges recorded from electrodes 1 and 8 (*A, B, D*) or only electrode 8 (*C, E, F*) of the 3+11 multielectrode. *A, B, C, F* are shown as single sweep recordings in Fig. 13. Calibration: 2 mV and 500 μsec.

in arrival time between the two spikes that determines the interspike distance.

An analysis of the mutual time interval between the two spikes at consecutive discharges of "double spikes" (Figs. 20, 21) revealed the following characteristics:

(*a*) There was an apparently random variability about the mean interval from discharge to discharge, i.e. the jitter phenomenon.

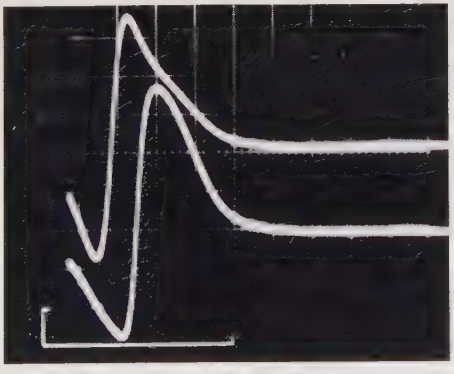

FIG. 19. *Superimposition of 58 consecutive action potentials* on one photograph. Recordings from electrodes 1 and 8 of the 3+11 multielectrode. The shutter was open for 5.6 sec. The time interval between consecutive discharges averaged 97 msec with a range of 70 to 180 msec. Calibration: 2 mV and 500 μsec.

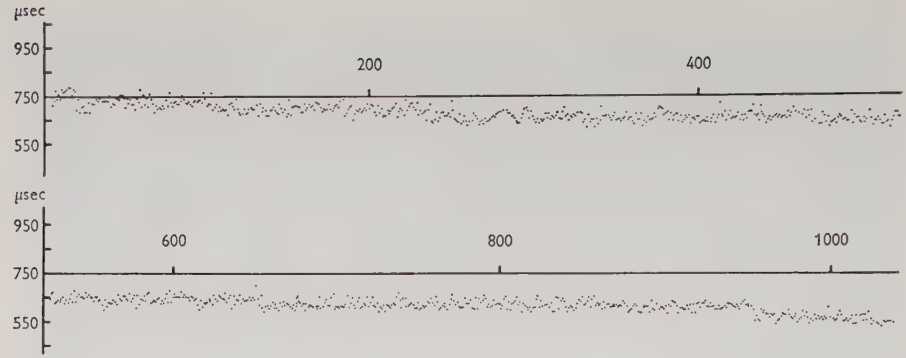

FIG. 20. *Inter-spike interval in consecutive discharges of double spikes.* The two diagrams are a continuous recording of about 1000 discharges over a period of approximately 100 seconds. *Abscissae:* discharge number. *Ordinates:* inter-spike interval. There was a gradual decrease in the mean time interval of about 200 μsec during the course of the experiment. The inter-spike interval measured between the baseline intersections of the rising phase of the spikes.

(*b*) Sometimes there was a more or less sudden change to an entirely new mean time interval.

(*c*) There were slow variations of the mean time interval. It was often observed (as in Fig. 21) that the mean time interval gradually increased or decreased (with up to a millisecond) and sometimes one of the spikes then suddenly disappeared.

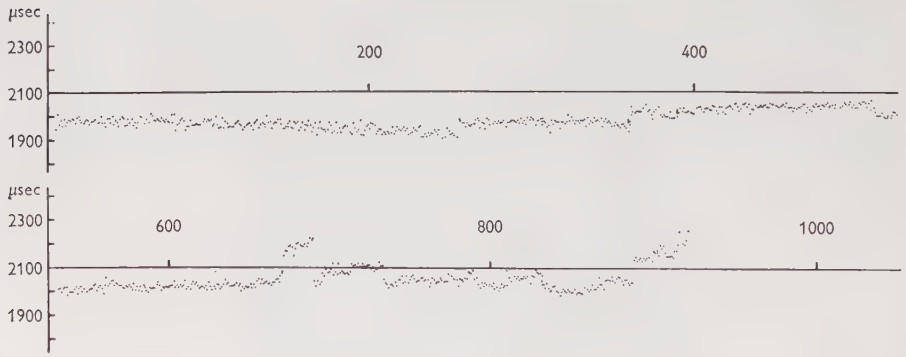

FIG. 21. *Inter-spike interval in consecutive discharges of double spikes.* The two diagrams are a continuous recording of about 850 discharges over a period of approximately 85 seconds. *Abscissae:* discharge number. *Ordinates:* inter-spike interval. There are irregular variations in the mean time interval. Towards the end of the recording the mean time interval rapidly increased until the second spike suddenly disappeared.

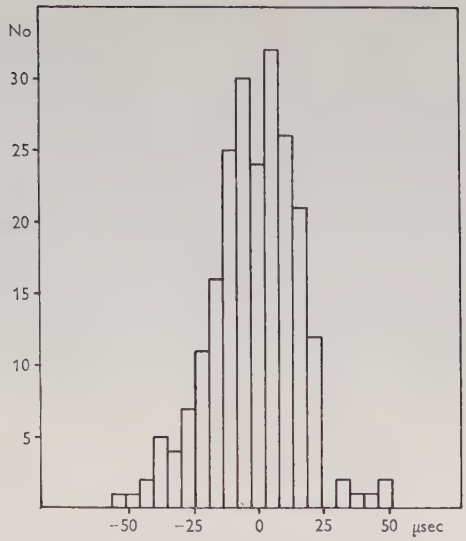

FIG. 22. *Distribution about the mean time interval* in consecutive discharges of double spikes. S.D. = 16.6 μsec. $N = 223$. During the period of measurements the mean time interval appeared to be constant.

In Fig. 22 is shown a histogram of the inter-spike interval during a period in a recording when the mean time interval was fairly constant. However, such a constancy was only exceptionally encountered. Therefore the standard deviation of the mean time interval could not be used as a measure of the jitter. However, it was the main purpose of this study to determine the single muscle fiber criteria and, in this respect, it was not the standard deviation of the time interval that was of interest, but the maximum variations during the period where the consecutive action potentials could be compared. As it was standard practice to make superimposition photographs of five consecutive action potentials for revealing the jitter, the following investigation was performed. Consecutive double spikes were recorded for several minutes. The material was divided into groups of five consecutive discharges. The *maximum and minimum* time difference between the two components, i.e. the range of variation within each series of five, was measured. The results of measurements on different double spike recordings are given in Table 1. The mean value found in this way will be referred to as "the jitter-value". The jitter-value was found roughly to correspond to twice the standard deviation of the mean time interval between the components when the mean was constant. The minimum jitter-value encountered was 10.6 μsec.

TABLE 1. *Jitter in "double spikes".*

All experiments made in the brachial biceps muscle. Recording made within the innervation zone in experiments 1–7, outside the innervation zone in 8–15 and close to the tendon in 16. The *jitter-value* is the range of variation within each series of five consecutive discharges. The inter-spike distance measured between the baseline intersections of the rising phase of the spikes.

Experiment	Standard deviation of mean time interval between the spikes (μsec)	Number of discharges	Jitter-value (μsec)	Standard deviation of the jitter-value (μsec)	Number of measurements
1	—	—	14.9	8.9	84
2	12.6	49	—	—	—
3	13.2	49	—	—	—
4	13.3	89	—	—	—
5	—	—	34.3	13.4	78
6	20.5	129	35.5	13.2	137
7	—	—	54.5	19.1	98
8	7.2	87	10.6	6.3	32
9	12.9	22	—	—	—
10	14.7	82	—	—	—
11	16.1	130	27.8	11.3	138
12	16.6	223	28.7	12.9	68
13	17.4	43	—	—	—
14	27.5	31	—	—	—
15	—	—	54.9	21.5	114
16	—	—	28.3	13.8	93

The possible causes of the jitter phenomenon. The jitter phenomenon might be attributed to the following possible factors:

1. Mechanical movements of the muscle fibers so that the position of the two fibers in relation to each other and to the needle was different in consecutive discharges.
2. Variability in the propagation velocity of the muscle fibers.
3. Variability in the propagation velocity of the terminal nerve fibers from the point of the last common bifurcation to the end-plate.
4. Variability in the end-plate delay, i.e. the time interval between the arrival of a nerve impulse to the nerve ending and the start of the depolarisation in the muscle fiber.
5. Variability in the starting point of the muscle fiber depolarisation due to the complex structure of the end-plate.

Ad. 1. External squeezing of the muscle firmly against the needle and manipulation of the needle did not appear to alter the size of the jitter, neither did gross variations in the length of the muscle. Varying the dis-

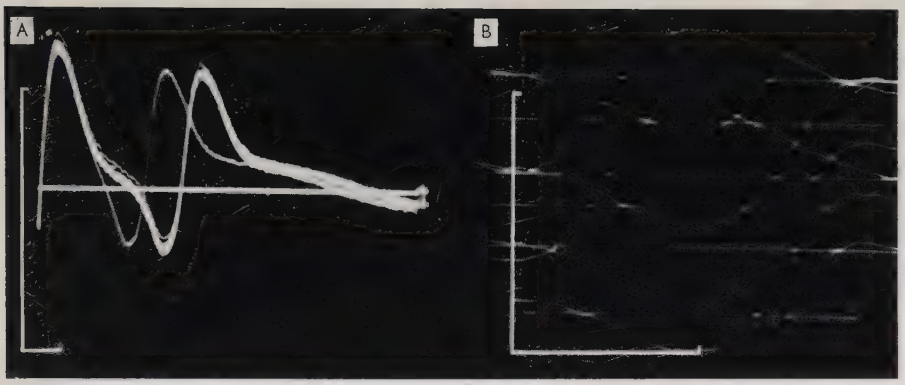

FIG. 23. *Double spike recording with bimodal distribution of the inter-spike interval.* In two discharges the time interval was 0.54 msec and in five 0.73 msec. In *A* the discharges are superimposed. In *B* the sweep is expanded five times so that the first spikes are outside the screen and the vertical position of the trace has been altered some centimeter downwards for each sweep so that each action potential and its respective baseline is seen isolated. Calibration: 2 mV and 200 μsec.

charge rate from a few up to 15 discharges per second did not seem to alter the jitter appreciably. As already mentioned, the shape of the individual spikes in a "double spike" were always equal from discharge to discharge (see Fig. 18). All these findings indicate that alterations in the mutual position of the fibers between consecutive discharges is not an important cause of the jitter phenomenon.

Ad 2. The variations in propagation velocity of the muscle fibers necessary to cause a jitter of the order of magnitude found here are exceedingly small. At a distance of 40 mm from the neuromuscular junction a difference in the propagation time from discharge to discharge of 100 μsec (a large jitter-value) is caused by a propagation velocity difference of only 1 per cent (which is just detectable by the multielectrode method while recording a single fiber from electrodes 1 and 8).

As a rule, there were slow variations in the propagation velocity and usually a continuous decrease over a period of 10–20 minutes (STÅLBERG, to be published). The slow variations in the mutual time interval might well correspond to an unequal decrease of the propagation velocity of the two fibers (see Figs. 20, 21). Therefore, it seems not impossible that also a short-time variability, appearing as a jitter, could exist. However, it could not be directly detected with the present accuracy of the methods. No such variations have been reported in the literature.

The order of magnitude of the jitter obtained within the innervation zone was about the same as that obtained outside the innervation zone as seen

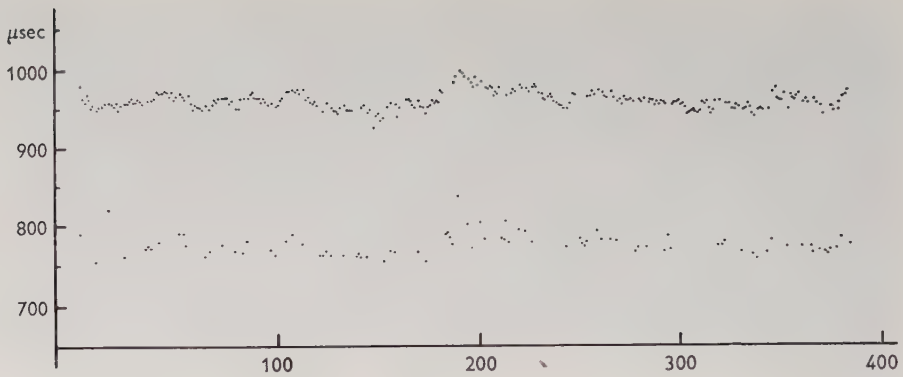

FIG. 24. *Inter-spike interval in consecutive discharges of the double spike shown in Fig. 23. Abscissa:* discharge number. *Ordinate:* inter-spike interval. About 350 discharges during 35 sec. The time intervals are grouped about two means (see page 55).

in Table 1. This finding suggests that the variability in the propagation velocity does not contribute appreciably to the jitter but the material is still too small to allow definite conclusions.

Ad 3 and 4. No experimental results have been obtained that can clarify the variability of the propagation velocity in the terminal nerve fibers and in the end-plate delay, and no information can be obtained from the literature.

Ad 5. Sometimes a bimodal distribution of the mean time interval between the components of a double spike was encountered. Such a case is shown in Figs. 23 and 24. 70 per cent of the discharges were grouped about a mean time interval of 1 msec, and 30 per cent about 0.85 msec. Usually, as in this case, one of the time intervals was more common. The phenomenon has been interpreted as being caused by double end-plates, as discussed on page 67.

Comment on the causes of the jitter. When recording within the innervation zone of a motor unit, the possible effects of a variability in the propagation velocity of the muscle fibers ought to be minimized, and the jitter in such experiments should mainly be due to factors located within the end-plate or in the terminal nerve fibers. Considering the highly delicate mechanisms that are involved in neuro-muscular transmission it seems reasonable to assume that *the main part of the jitter phenomenon has been caused by a variability in end-plate delay.* (Note, that it is the variability in the delay in *two* independent end-plates that determines the size of the jitter.)

The findings under point 5 that might have been caused by double end-plates pose the question if part of the jitter could possibly be caused by a

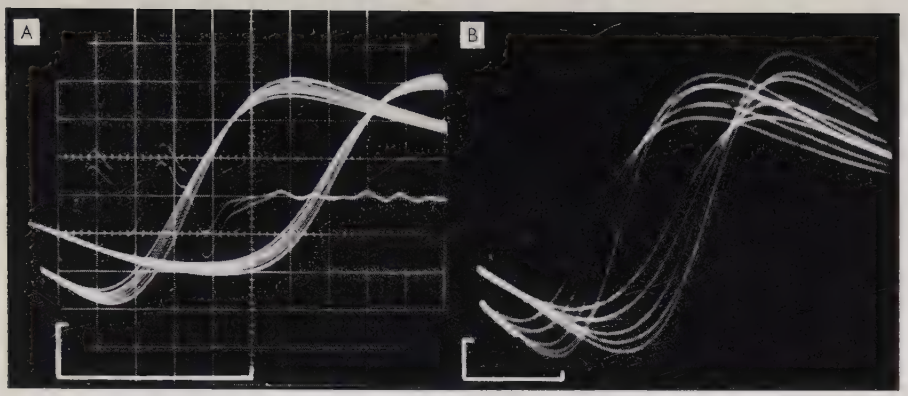

FIG. 25. *Movement artefacts* obtained by moving the needle to-and-fro during exposure. Electrodes 1 and 8 of the 3+11 multielectrode. Calibration: 2 mV and 100 μsec.

similar mechanism also within each end-plate. This is further discussed on page 67.

The jitter phenomenon and the slow variations in the inter-spike intervals need further investigation.

The jitter between the action potentials of adjacent fibers. Special interest was given to the case when the action potential sources were shown to be close to each other, i.e. when the two component action potentials had their maxima on adjacent electrodes (or even closer), had about equal size and were simultaneous enough to interfere with each other in the trough, rising phase or peak. This occurred only rarely. In the five cases that were studied (Fig. 17 *A, C, J, L, M*) the jitter does not seem to have been less pronounced than when the component sources were more remote from each other.

If the shape of the action potentials from electrodes 1 and 8 differed due to different propagation velocities of the components, the jitter was seen in separate parts of the two potentials (Fig. 17 *A, B, C, E, G, H, K*).

The "technical jitter". It must once more be stressed that the jitter in a composite potential appeared as variations in a *part* of the display. This is distinct from the variations in shape caused by changes in the electrode position which affect the whole of the action potential (Fig. 25). The superimpositions of action potentials from other motor units only produced occasional variations in the shape. A 50 cps A.C. interference from the mains produced an unstable oscilloscopic display due to random fluctuations of the trigger level which also affected the whole of the waveform, and was

easily recognizable. Any instability in the horizontal deflection circuits of the oscilloscope that might give something resembling the "action potential jitter" can definitely be excluded (see page 29).

Simulation experiments

The actual jitter could not be simulated with the present methods. An estimate of the limits within which the shape variations of the action potentials would occur was obtained from the experiments described on page 44, where, instead of comparing consecutive action potentials, as under ordinary conditions, two simultaneous potentials were compared (Fig. 14 is an example).

When the two component action potentials were of approximately equal voltage and shape and their mean time interval was about zero the jitter took the form of a tilting of the rising phase about the trough. The peak and the trough also underwent small variations in shape. The trough was locked in its position by the triggering, which was the reason for the apparent tilting about the trough. With increasing mean time interval the phenomenon became confined to only the negative phase of the potential. When the mean interval exceeded the duration of the rising phase, the component action potentials became individually discernible (as in the actual recording in Fig. 17 *C*).

When one of the components was considerably smaller than the other, the appearance of the jitter was, in principle, the same but quantitatively less pronounced. The case when the jitter seemed to be least apparent was when the component action potentials were nearly simultaneous.

It must be mentioned that the jitter under ordinary recording conditions could be considerably modified by the capacitive cross-talk between the electrodes, as seen in Fig. 17 *K*.

It was found that a difference of 1 mm between any part of consecutive potentials was detectable both upon direct inspection of the screen and indirectly from the photographs when using the superimposition techniques. This corresponds to 2 μsec at a sweep of 20 μsec/cm. As the minimum jitter value encountered was 10 μsec it can be concluded that the jitter in a composite action potential, where the two components are of about equal amplitude, would be detected under all circumstances.

To study the situation when the components were of grossly different amplitude the most unfavourable case was chosen, namely, when the two components were nearly simultaneous. The 3+11 multielectrode (Fig. 1, page 17) was used. A clean, smooth action potential with a propagation time of 40 μsec between electrodes 1 and 8 was selected. Electrode 3 (at

FIG. 26. *Simulation of jitter.* See page 58. Appearance of the composite potential when two spikes are added without time interval (the clean spikes) and with the main spike leading by 40 μsec. In *A* the smaller spike is 50 per cent of the main spike; in *B* 10 per cent and in *C* 5 per cent. Recording with two different sweep velocities. Calibration: 2 mV and 100 μsec.

one end of the row) was made to overlie the active fiber with the electrode row perpendicular to it. The action potentials from electrodes 8 and 1 then equalled each other in shape; the former was simultaneous with the action potential from electrode 3, the latter lagged by 40 μsec. Both were less than 1 per cent of the amplitude from electrode 8. In one amplifier channel the signals from electrodes 3 and 8 were added, in the other those from electrodes 3 and 1. The traces were vertically aligned on the oscilloscope. No differences between these two potentials could be detected. The interfering

potential was then increased by repeating the procedure with electrode 4 overlying the fiber and adding 4 and 8 on one channel and 4 and 1 on the other and so on.

From two experiments of this type it was concluded that the presence of a second fiber with a jitter value of 40 μsec will be detected if the amplitude of its action potential exceeds 5 per cent of the main action potential (Fig. 26).

Under actual conditions the jitter appears as a movement of a part of the display. Because of the sensitivity of the eye for movement, it is possible that the static simulation experiments underestimate the sensitivity of the procedure for detecting an interference.

The action potentials from the different electrodes in the long row

The single muscle fiber criterion: The action potentials recorded from the different electrodes in the long row have simultaneous baseline intersections and a steep and symmetrical voltage decrement with increasing distance (see Fig. 34).

This criterion was not fulfilled by the actual composite potentials that were studied.

Simulation experiments

It must be borne in mind that the voltage decrement only refers to the distances along the long electrode row in the multielectrode and not to the real distances between source and electrode, which are unknown. Therefore, no absolute measured quantity in the decrement curve could be used as a single fiber criterion, only the general shape of the curve.

To investigate the shape of the curve for the case when two identical fibers with simultaneous propagation were positioned beside each other in relation to the multielectrode one simulation experiment was performed according to Fig. 27. A voluntary action potential was chosen, fulfilling the single muscle fiber criteria and having a decrement curve similar to that from a chemically activated single fiber and without signs of interference on any electrode. Recording was first made according to the procedures described on page 24, with mutual recording distances along the needle of 30 μ, so that 22 recording sites were obtained. The same procedure was then repeated but the signals from two adjacent electrodes were added at each recording. The decrement curve thus obtained equals the one that would have been obtained when recording in an ordinary way from two real fibers lying beside each other, as seen from the electrode, with a center-to-center distance of 50–60 μ (the individual interelectrode distances are not absolutely equal).

In Fig. 27 is shown the original voltage decrement curve and that for the

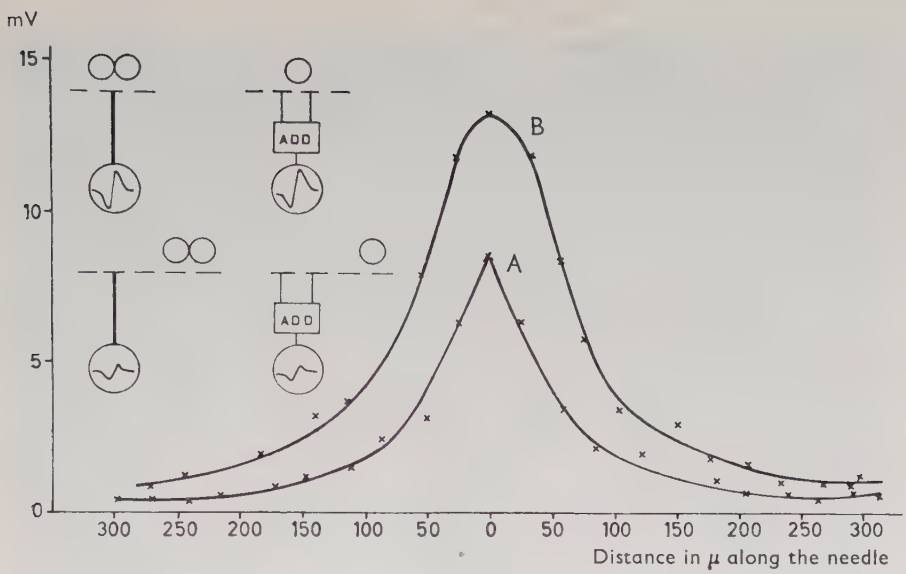

FIG. 27. *Voltage decrement curves.* See page 60. Spike voltage plotted against distance along the needle. Curve *A* is obtained with ordinary recording procedures. Curve *B* is obtained by adding the potentials from two adjacent electrodes as shown in the right part of the schematic drawing and it simulates the curve that would have been obtained with two equal fibers positioned beside each other with a center-to-center distance of 50–60 μ (the left part of the schematic drawing).

simulated composite potential. On either side of the electrode of maximum amplitude (8 mV) the original curve was a continuously decreasing function but the curve for the simulated composite potentials was ∫-shaped and had an inflection point.

The diameters of the muscle fibers in the biceps muscle have been measured by BUCHTHAL *et al.* (1955 *a*). In an adult muscle from a 62-year-old woman the mean value was 48 μ and the lowest value 25 μ. This implies that two fibers of average diameter, with simultaneous action potentials, lying closely beside each other relative to the needle and at such a distance from the needle as to make each of maximum amplitude 8 mV, would be detected by the shape of the decrement curve.

The case with one fiber positioned beside and behind another one in relation to the multielectrode has been constructed from two experimentally obtained decrement curves, one with maximum amplitude of 8 mV over electrode 8 and the other with maximum amplitude of 6 mV over electrode 7. The lack of symmetry in the curve was obvious.

However, when two fibers with simultaneous propagation are positioned exactly behind each other in relation to the multielectrode, the decrement

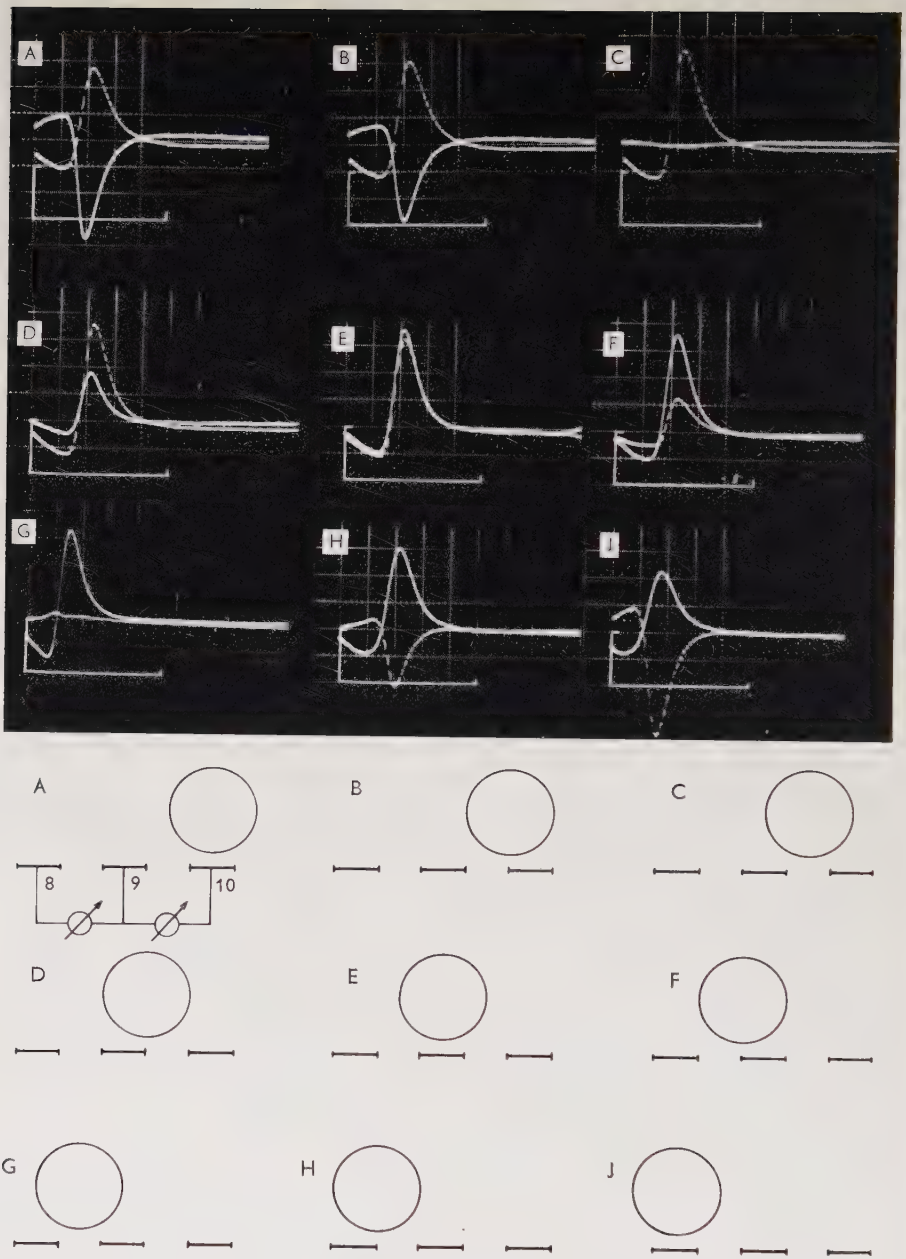

FIG. 28. *Recordings fulfilling the bipolar single fiber criterion.* The schematic drawing shows the electrode–fiber position for each recording. Note that one of the action potentials is nearly zero in *C* and *G*. In *E* the two action potentials equal each other *and* are both at maximum voltage. The 3 + 11 multielectrode. The dotted traces from electrodes 9–8. Calibration: 2 mV and 500 μsec.

curve ought to be a continuously decreasing function, symmetrical on both sides of the electrode of maximum amplitude. This condition is thus not detectable by the decrement curve.

The fiber chosen for this experiment must have been relatively close to the needle because the action potential voltage was relatively high. At increasing recording distance the decrement curves become flatter (see Fig. 34) and the ∫-shape must be more difficult to detect. It must also be emphasized that several fibers that are arranged in a round bundle *and* have simultaneous propagation ought to give a decrement curve with a perfectly "sharp top". If the shape only of the decrement curve is taken into account the present criterion is only a test of symmetry of the action potential source and says nothing about its size.

The study of the shape and symmetry of the decrement curves is more conveniently made in the bipolar than in the monopolar recordings (see below).

The bipolar recordings

The single muscle fiber criterion: The bipolar recordings 9–8 and 9–10 can be made equal and simultaneous, and this occurs in that needle position when they are both maximum. The monopolar action potential from electrode 9 is then also maximal. By to-and-fro adjustment of the needle position either of the bipolar potentials can be made successively smaller down to zero. The base-line intersections for all these action potentials are simultaneous (Fig. 28).

Actual composite potentials

As expected, this proved to be a most sensitive test for revealing composite potentials. The criterion was never fulfilled by the composite potentials. It often proved possible to cancel one of the components by appropriate positioning of the multielectrode, and thus to discern the other component more accurately. This property of the recordings is explained in more detail below. Thus, the jitter phenomenon was often more clearly visualized in the bipolar recordings, especially when the components were nearly synchronous.

Simulation experiments

The bipolar recordings from a two-fiber-generator were difficult to simulate. However, from the experimentally obtained monopolar decrement curves in Fig. 27 the *bipolar decrement curves* could be geometrically constructed and are shown in Fig. 29. In *A* (the single fiber generator) the bipolar recordings are at their maximum amplitude in the same position of the multielectrode, and the bipolar action potentials are then of equal voltage. In *B* is shown the bipolar decrement curves for the simulated case of two fibers beside each other, with a center-to-center distance of 50–60 μ.

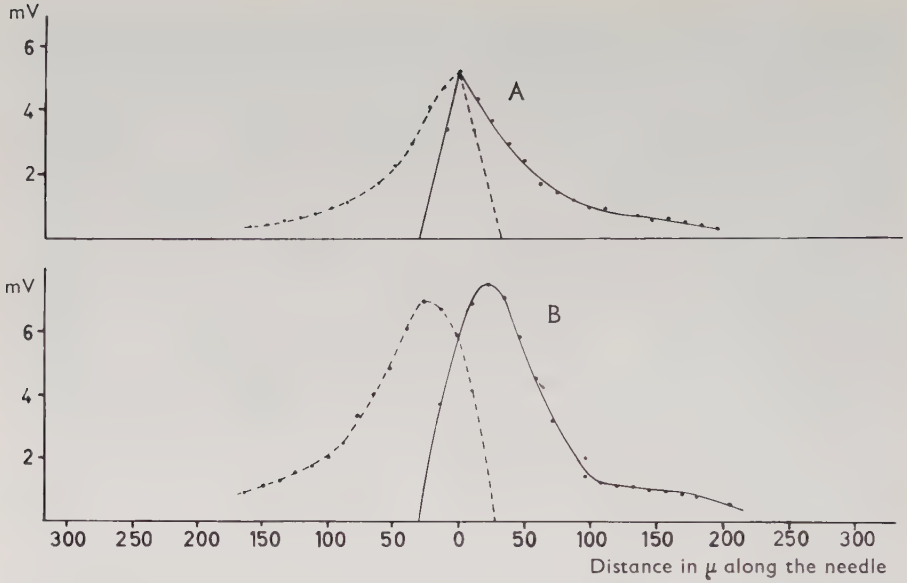

FIG. 29. *Bipolar voltage decrement curves* constructed from the monopolar curves in Fig. 27. *A* shows the curves obtained from a single fiber with electrode combinations 9–8 (dotted lines) and 9–10 (full lines). The curves have coincident maxima. *B* shows the curves for the simulated case with two fibers beside each other. The maxima of the two curves in *B* are separated by 60 μ. All distances have been counted from the electrode that is common for the two bipolar pairs (electrode 9).

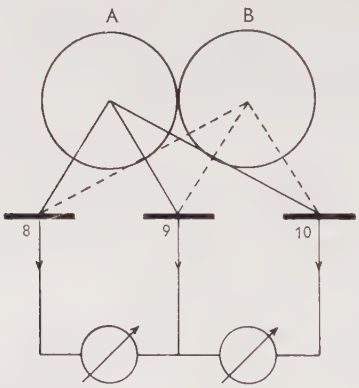

FIG. 30. *Method of cancelling fiber action potentials by bipolar recordings.* The idealized case with two 60 μ fibers closely beside each other. Bipolar recording; electrodes with a center-to-center distance of 60 μ. The action potential from fiber *A* is cancelled by bipolar recording from electrodes 9–8 and from fiber *B* by bipolar recording from electrodes 9–10. Thus, electrodes 9–8 record the action potential from fiber *B*, and electrodes 9–10 the action potential from fiber *A* exclusively.

The two bipolar recordings have their maxima separated. Little doubt exists that the composite character should be revealed in this case.

If the multielectrode should be positioned in such a way in relation to two adjacent fibers as shown in Fig. 30, the right bipolar recording should be dominated by the left fiber and the left bipolar recording by the right fiber. Then even a slight asynchrony in the components of the order of 2 μsec should be detected by the non-simultaneity of the potentials. Triggering the sweep from one of the bipolar potentials should make the jitter appear as to-and-fro displacements of the other bipolar potential.

The case when two fibers are positioned strictly behind each other is not detectable as any asymmetry or separation of the maxima.

The bipolar recordings, as used here, are useful as tests of the symmetry of the action potential source but give no information as to its size. The sensitivity in the detection of an unsymmetry decreases with increasing recording distance.

Recordings fulfilling the single muscle fiber criteria

Action potentials that fulfilled the criteria for single muscle fiber potentials in every detail were found frequently in the voluntarily activated muscle. However, it was a common experience to find interference from other fibers of the motor unit when recording from the outer electrodes in the long electrode row. It was, however, usually not difficult to construct the individual decrement curves for both the fiber overlying the central electrode and the interfering fiber so that the voltage of the action potential from the interfering fiber could be calculated for the central electrode. An unsymmetrical decrement curve, when the cause is thus analyzable, can still be compatible with a single fiber recording. However, it sometimes occurred that the decrement curve was unsymmetrical for other reasons. When, for instance, an action potential was found immediately after the needle had pierced the fascia, felt as a tough resistance, a number of electrodes could be within and the others outside the fascia, making recording conditions different in the two parts of the electrode row.

It is also possible that there may be inhomogeneities within the bulk of the muscle that could influence the action potential decrement in a similar way. This question will be further discussed in Chapter 4.

No recording was accepted if the decrement was not strictly symmetrical over the three central electrodes, as checked with the bipolar recordings.

There were considerable differences in different muscles as to the frequency of occurrence of simple potentials. In the brachial biceps or frontalis muscles, potentials fulfilling the criteria were easily found, usually at each

insertion of the needle, but in the tibialis anterior and gastrocnemius muscles such recordings were only rarely encountered. In the extensor digitorum muscle of the arm and the opponens pollicis muscle the recordings were often polyphasic with the individual spikes clearly discernable. There were also considerable differences between the same muscle in different subjects; potentials fulfilling the criteria being more easily found in the gross muscles of men than in the thin muscles of women or children. There were also differences in this respect between subjects having muscles of approximately the same mass.

The shape of the action potentials will be reported and discussed in Chapter 4 in conjunction with the distortion factors that have been introduced by the method.

Discussion

The jitter phenomenon

The jitter phenomenon does not seem to have been described in earlier literature. In human electromyography, when using ordinary concentric needle electrodes, it is clearly seen (page 83), but tends to be obscured by the instability in the wave form that is caused by movements of the electrode in relation to the fibers. Such movements are difficult to avoid when the electrode is on the bevel surface of the needle tip. The jitter is difficult to notice when using a free running sweep on the oscilloscope, as is usually the practice.

The jitter phenomenon has not been described in animal nerve-muscle preparations.

It has been postulated (page 56) that the action potential jitter is mainly due to a variability in the end-plate delay, i.e. the time interval between the arrival of a nerve impulse to the nerve-ending and the start of the propagated depolarisation in the muscle fiber. In the frog nerve-muscle preparation, kept at 20°C, the end-plate delay is 1.1–1.3 msec. It increases at partial curarization and at fatigue (KUFFLER, 1944). In the human end-plate at 37°C, the end-plate delay seems to be unknown but is probably shorter.

There are reasons to believe that the jitter is largely influenced by random factors. The jitter-value corresponded to roughly two times the standard deviation (S.D.); it is to be expected that 2 out of 6 consecutive intervals should fall outside the range ± 1 S.D., which is in good accordance with the experimental results (Table 1).

With the assumption that the jitter is the variability in the end-plate delay of *two* end-plates, and that the variability for each end-plate is equal, the

individual variability, expressed as the standard deviation, is $1/\sqrt{2}$ of the variability of the inter-spike interval and is hence of the order of magnitude of 5–20 μsec.

The occurrence of a bimodal distribution of the inter-spike distances in consecutive double spikes in the case illustrated in Figs. 23, 24, affords an interesting interpretation. About 2 per cent of the muscle fibers in human muscle are known to have double end-plates innervated by the same neurone (Coërs & Woolf, 1957). If the impulse in the muscle fiber should start alternately from the two end-plates there ought to be a bimodal distribution of the difference in the arrival time of the action potentials. Now it is reasonable to ask if part of the apparently random variations about the mean in fibers with a single end-plate could be due to a similar mechanism. The end-plate is not a "plate" but has a highly complex branching pattern and it can be imagined that initiation of the propagated impulse from different parts of this structure at different occasions could be partially responsible for the jitter. However, the mechanism involved in neuromuscular transmission, i.e. setting free of the acetylcholine at the arrival of the nerve impulse, the depolarisation process of the end-plate, the initiation of the propagated impulse and the inactivation of acetylcholine and recovery, is so complex that some variability in neuromuscular transmission time is highly probable.

It seems that not only the jitter phenomenon but also the slower variations in the inter-spike interval that have been found (page 51) could be of interest for the study of end-plate physiology and pathophysiology. In myasthenia gravis, where neuromuscular transmission is directly affected, the conditions ought to be different from the normal, as in certain myopathies, where the end-plates are known to be malformed and spread over great lengths of the fibers, and also in denervation and subsequent regeneration by collateral sprouting from other axons. Also investigations of the jitter under influence of neuromuscular-active drugs ought to be of interest.

The single fiber criteria

The possibility that two fibers at about the same distance from the electrode should have been the generator of a recording that fulfils the single muscle fiber criteria seems to be most unlikely. The most important proof for this statement is the presence of the jitter phenomenon that ought to reveal such a composite potential under all circumstances. But also if the jitter is left out of account the remaining criteria seem to offer good evidence in support of this statement.

What conditions are necessary for two *adjacent* fibers to generate a com-

pound action potential that fulfils the criteria? As to the spatial arrangement it is unlikely that they could be positioned beside each other, because this should reveal itself as a blunt appearence of the decrement curve (page 60) together with a separation of the sites of maximum amplitude for the two bipolar recordings (page 63) (at least if the fibers are so close to the electrode as to make their individual action potential 8 mV or more). If one fiber should be behind and beside the other there should also be an asymmetry in the decrement curve and in the bipolar recordings (page 61). The two fibers must then be positioned behind each other in relation to the multielectrode (page 61) to escape detection.

In order that the composite action potential should be perfectly smooth and clean in shape it is necessary that the arrival time of the two component action potentials be simultaneous to within 30–60 μsec (page 47). If the two fibers should have exactly the same site of initiation of the impulse, i.e. the same location of the end-plates, and the recording is made at a distance of 35 mm from the site, and assuming a propagation velocity of 3.5 meter/sec for one fiber, then the other must have a propagation velocity of 3.5 ± 0.01–0.02 meter/sec to mask the composite character. In other words, the propagation velocities must be equal to within 0.3–0.6 per cent. If the two fibers should have different locations of their end-plates, there must be a corresponding difference in the propagation velocities to within 0.3–0.6 per cent to make them simultaneous at the site of recording. This ought to happen only very rarely, provided that there is no synchronization between the fibers. Such a synchronization is assumed by BUCHTHAL *et al.* (1957 *a*) to explain the smooth, clean shape of the subunit potential in spite of its being generated by up to 30 fibers of different diameters and with different sites of innervation. No evidence for any form of synchronization has been found in the present investigation (unequal propagation velocity of adjacent fibers, page 47, and jitter between adjacent fibers, page 57, are not consistent with a synchronization process). Synchronization is further discussed in Chapter 6 on page 87.

Thus, it can be concluded that, *two adjacent fibers are highly unlikely to give a recording that fulfils the single muscle fiber criteria.* Hence, several fibers in a bundle can definitely not give such a recording.

It was stated in the introduction to this chapter that there must be an interference from the rest of the motor unit when recording from one muscle fiber. The possibility of detecting an interference from a remote fiber depends on the jitter-value, the mean time interval between the action potentials of the main and the interfering fiber and the position of the fibers in relation to the electrode. It was found experimentally (page 58) under the unfavorable conditions when the interfering fiber lay exactly

behind the main fiber and the mean time interval between their spikes was nearly zero, that the voltage of the interfering spike potential must exceed 5 per cent of that of the main spike in order to be detected, provided the jitter-value exceeded 40 μsec. *Assuming* the same conditions, a reasonable estimate would be that if the jitter-value should be 20 μsec, the interfering voltage must be 10 per cent to be detectable, and with a minimum jitter-value of 10 μsec it must be 15 per cent. Since most jitter-values were above 20 μsec an interference from one spike with amplitude 10 per cent of the main spike should be detected whereas interference from a spike smaller than 5 per cent may easily be missed.

However, it is possible that *several* fiber potentials, each of low amplitude, might summate to give an interfering composite potential. Even here the jitter phenomenon ought to be visible and there are no reasons why the jitter should be less pronounced in such a case than with an interfering single spike.

Concluding remarks on the single muscle fiber criteria

The investigation has shown, that *spike potentials that were of identical shape at consecutive discharges were single muscle fiber potentials.* This must be true when using any type of recording system provided the time resolution is sufficient to permit the detection of a variability in a part of the wave form of the order of 10 μsec. According to the experience gained in the present investigation no criterion besides absence of jitter is needed. This implies that all the electrodes in the multielectrode are by no means necessary for the selection of single muscle fiber potentials, as they can be as efficiently selected with only one of them. However, it is necessary that the electrode position in relation to the fiber be kept fixed in order that no movement artefacts obscure the jitter.

CHAPTER 4

PARAMETERS OF THE SINGLE MUSCLE FIBER ACTION POTENTIALS WITH PARTICULAR REGARD TO THE DISTORTIONS

The extracellular action potential represents the potential variations in a point outside the muscle fiber caused by the electrical events over the muscle fiber membrane. The shape of the action potential is determined by the properties of the muscle fiber, the properties of the volume conductor surrounding the fiber and by the distance between the fiber and the electrode. Furthermore, some distortion factors are inevitably introduced by the recording system. Apart from these physical distortions there are also "statistical distortions" affecting, for example, the size distribution.

In this chapter the single muscle fiber action potentials from the material in Chapter 3 are discussed with particular regard to the distortions. The aim is, however, more to indicate the sources of distortion rather than to make a quantitative evaluation of them. An extensive review of this subject is that of BURKHARDT (1957).

Results

The single muscle fiber action potentials

The single muscle fiber action potentials obtained during voluntary contraction had similar shape to those obtained during chemical activation (Fig. 10, page 38). They had a smooth, biphasic spike-component, often followed by terminal phases of low amplitude and long duration (Fig. 31). The positive phase of the spike was usually of 10–20 per cent lesser voltage than the negative phase. Occasionally the positive phase could be absent or small (Fig. 31 *C*), considered by BUCHTHAL (1961, 1962) to occur when recording near the end-plate.

About 300 action potentials from 28 of the experiments made in the brachial biceps muscle of 15 young men and 4 young women have been measured. All action potentials have been obtained after adjustment of the electrode position for optimum spike voltage. The spike voltage, rise-time

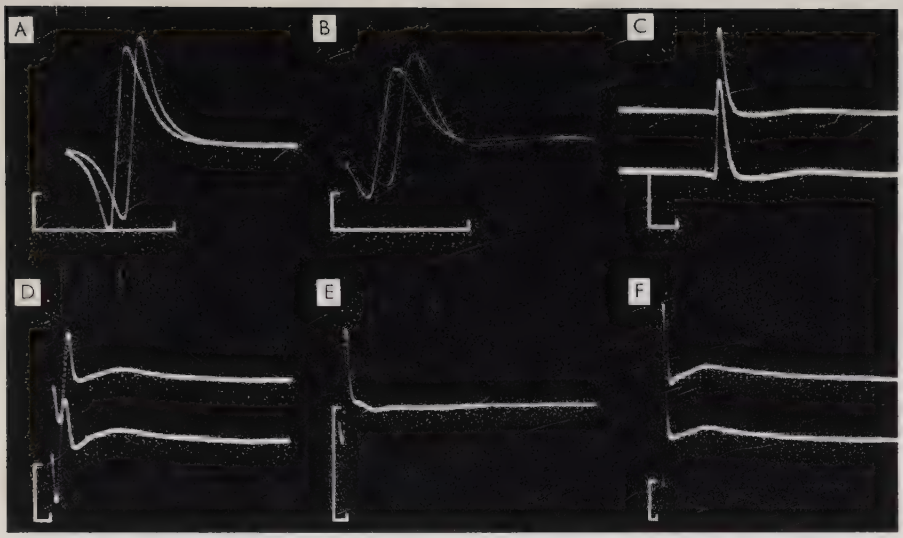

FIG. 31. *Examples of single muscle fiber action potentials.* The 3 + 11 multielectrode. *A, B, D, F* from electrodes 1 and 8, *E* from electrode 8. Both potentials in *C* from electrode 8 and probably recorded near the end-plate, the upper with pass-band 60–35,000 cps and the lower with 5–35,000 cps. The potentials in *D* with pass-band 5–35,000 cps. The other potentials with pass-band 60–35,000 cps. Calibration: 2 mV and 500 μsec.

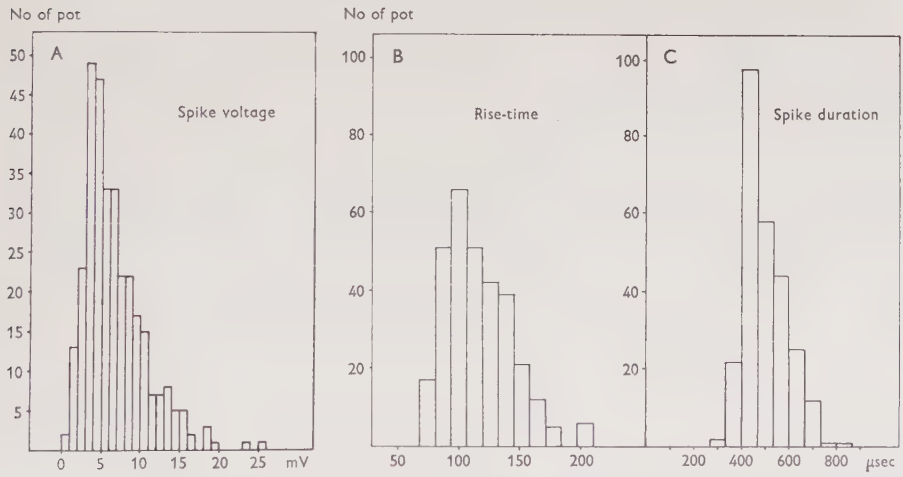

FIG. 32. *Spike voltage, rise-time and spike-duration* for single muscle fiber action potentials (measured according to Fig. 7). *A, Spike voltage:* median value 5.6 mV. range: 0.7–25.2 mV. $n = 309$. *B, Rise-time:* median value 112 μsec. range: 67–200 μsec. $n - 310$. *C, Spike-duration:* median value 470 μsec. range: 265–800 μsec. $n = 262$.

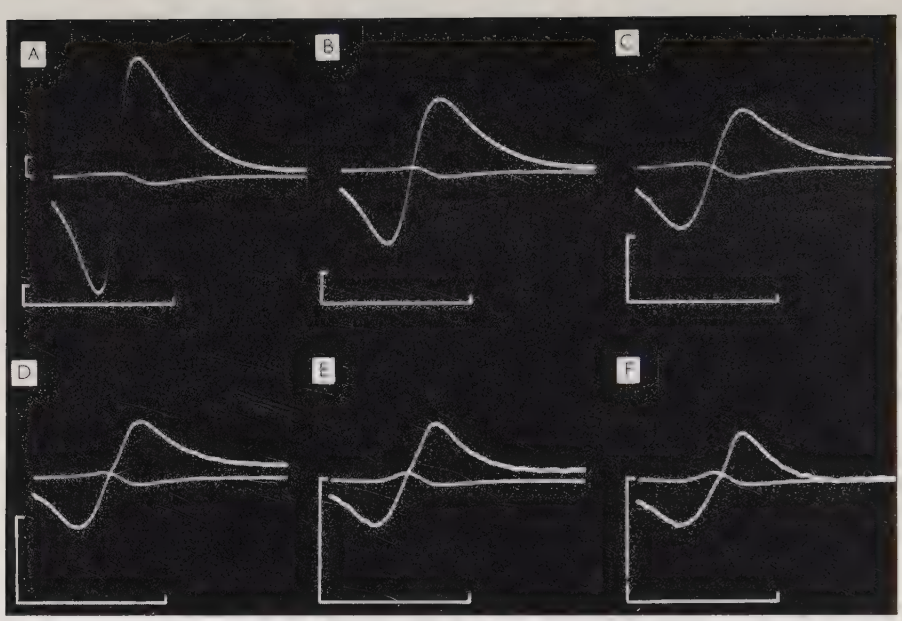

FIG. 33. *Recordings obtained at different distances from a fiber.* Electrodes 8–3 (*A–F*) of the 3+11 multielectrode with electrodes 1 and 8 aligned with the fiber. The bipolar lead-off 7–9 is used as a reference in each recording. There is a considerable cross-talk component from electrodes 1 and 8 in *D, E, F.* Calibration: 2 mV and 500 μsec.

and spike-duration are shown in Fig. 32. The three distribution curves show a positive skewness. The median value for the voltage was 5.6 mV, for the rise-time 112 μsec and for the duration 470 μsec.

Thirteen action potentials (4.2 per cent) were above 15 mV, with maximum value 25.2 mV. Ten of these recordings were obtained from male subjects with markedly well-developed muscles.

Five voltage decrement curves (i.e. the spike voltage plotted against the *distance along the needle*) are shown in Fig. 34. The shape of the action potentials in such an experiment is shown in Fig. 33.

Discussion

The distortion factors

The insulating wall around the electrodes

The action potentials were not recorded in an unlimited volume conductor but at the boundary of a volume conductor and an insulator; namely, the surface

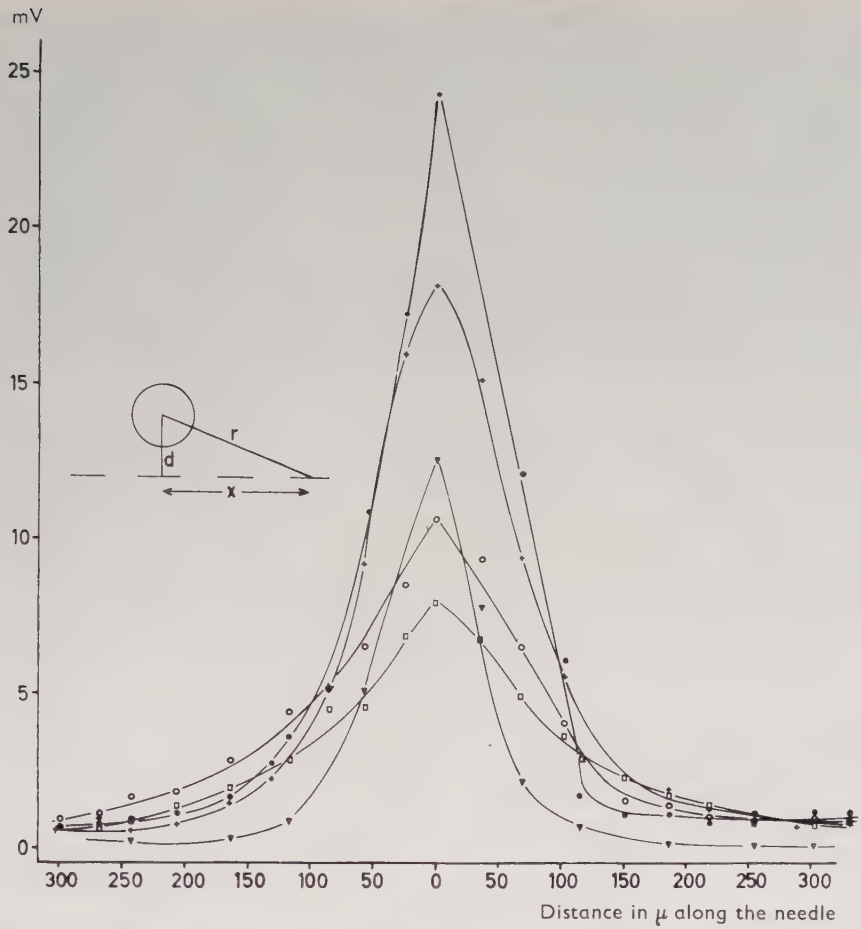

FIG. 34. *Voltage decrement curves.* Spike-voltage plotted against the distance along the electrode array of the 3 + 11 multielectrode (x in the inset). The fiber–needle distance (d) and the fiber–electrode distance (r) are not known. The plateaux in the outer parts of some curves are to be attributed to the cross-talk component (page 75). Distance "0" corresponds to electrode 8.

of the epoxy resin mould in which the electrodes were embedded (Fig. 1, page 17). By means of the "method of electrical images" (see e.g. JEANS, 1933, page 185; STRATTON, 1941, page 193; WEBER, 1950, page 215) it can be calculated that the effect of introducing an infinitely large insulating boundary in the plane of the electrodes, here called "the wall effect", will be to increase any voltage by a *factor of two* in comparison to that which would have been obtained in an unlimited volume conductor. Thus the action potential shape is not distorted by the presence of the insulator but the trough-to-peak voltage is doubled.

(This result has been confirmed in an electrolytic tank muscle fiber analog, where it was also found that the insulator surrounding an electrode must be of

at least twice the diameter of the muscle fiber to exert approximately the full wall effect. EKSTEDT & STÅLBERG, unpublished.)

The presence of the 14 metallic electrodes in the insulator might modify the wall effect and introduce some form of distortion that cannot be determined at present.

The metallic needle

The electrodes in the 3 + 11 multielectrode are somewhat unsymmetrically mounted in the opening of the needle (Fig. 1). Electrode 1 is only 70 μ away from one border of the opening while electrode 8 is 270 μ away. In spite of this there seems to be no other inequality between the action potentials from electrodes 1 and 8 (when these are aligned with the fiber) than that explained by the cross-talk (see below), neither when the impulse passes in the direction from electrode 1 to 8, nor when it passes in the reverse direction.

Thus it is reasonable to assume that the metallic needle is too remote from the electrodes to introduce any appreciable distortion.

The electrode area

If the electrode is not a point but has a certain area, it records the mean voltage distributed over its surface (HÅKANSSON, 1956).

Besides this, there is another factor that might be of some importance but does not seem to have been taken into account in the literature. The platinum surface is polarizable (BUCHTHAL *et al.*, 1954 *a*; BURKHARDT, 1957). Thus it is a good conductor for high frequency currents but a poor conductor for low-frequency currents. Therefore it is possible that the high frequency components of an action potential are decreased more by the area of the electrode due to electrical shunting of the currents in the volume conductor than the low frequency components. It is even possible that the low frequency components are enhanced; the polarized electrode surface exerting a "wall effect" (page 72). (This has been shown to occur for polarized electrodes in the electrolytic tank muscle fiber analog. EKSTEDT & STÅLBERG, unpublished.) However, no quantitative estimate of this effect can be made at present.

The electrode polarization also causes a low frequency *attenuation* due to the voltage division between the polarized electrode surface and the amplifier input impedance (see e.g. BURKHARDT, 1957). This effect is responsible for the limitation of the low frequency response of the electrode–amplifier system at 1 cps. (See page 29.)

A rough estimate of the influence of the electrode area is obtainable from the experiment shown in Fig. 35. With electrodes 1 and 8 of the 3 + 11 multielectrode aligned with a fiber, the effect of increasing the extent of the recording surface *along* the fiber was studied by connecting electrodes 1 and 8 (Fig. 35 *A*). This increased the rise-time with an amount equal to the propagation time between electrodes 1 and 8 but the voltage of the potential was only little affected. When connecting seven electrodes transversely to the fiber (5–11) (Fig. 35 *B*) the rise-time was mainly unaffected but the amplitude decreased. It may be permissible to draw the analogy that the extent of the 30 μ electrode *along* the fiber has increased the rise-time with an amount corresponding to not more than the propagation time across the electrode. With a propagation velocity of 3.5 m/sec

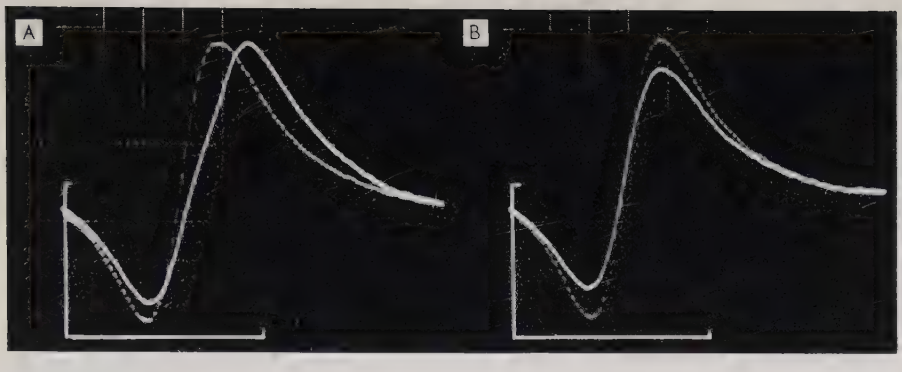

FIG. 35. *The influence of the electrode area on the action potential shape.* The 3 + 11 multielectrode with a fiber aligned with electrodes 1 and 8. The dotted action potentials in *A* and *B* (voltage 3.6 mV) obtained from electrode 8. The other potential in *A* has been obtained by connecting electrodes 1 and 8, and thus increasing the extent of the recording surface *along* the fiber. The rise-time is increased by an amount equal to the propagation time (80 μsec) over the combined recording surface but the voltage is not affected very much. In *B* an increase of the transverse extent of the recording surface has been obtained by connecting all electrodes from 5 to 11 (370 μ). The rise-time is hardly affected but the voltage decreases. Calibration: 2 mV and 500 μsec.

this should be about 10 μsec. The *transverse extent* of the electrode ought to decrease the potential voltage, but the amount of decrease must depend on the fiber diameter in relation to the electrode diameter and on the distance between fiber and electrode, which are unknown in any individual case. Therefore any quantitative estimate of the voltage decrease introduced by the 30 μ electrode cannot be made at present.

The cross-talk between the electrode leads

It has already been pointed out in Chapter 1 (page 17) that there was a capacitive cross-talk between the electrode leads. Some examples of the appearance of the cross-talk under different conditions are shown in Fig. 36. When recording from the outer electrodes in the 3 + 11 multielectrode, the cross-talked signal could often exceed the amplitude of the volume-conducted action potential. The plateaux in the outer parts of the decrement curves in Fig. 34 are due to the cross-talked signals, which were usually between 3 and 5 per cent of the amplitude of the largest action potential.

The cross-talk could be reduced by the use of electrode 3 or 13 as reference, but this has rarely been used in this investigation as it made the procedures too tedious (page 20).

Mechanical effects of the needle

The needle multielectrode has approximately 100 times the cross-sectional area of a single muscle fiber. A great many fibers must be crushed by the needle. It has already been stressed (page 16) that when the needle tip was blunt or was

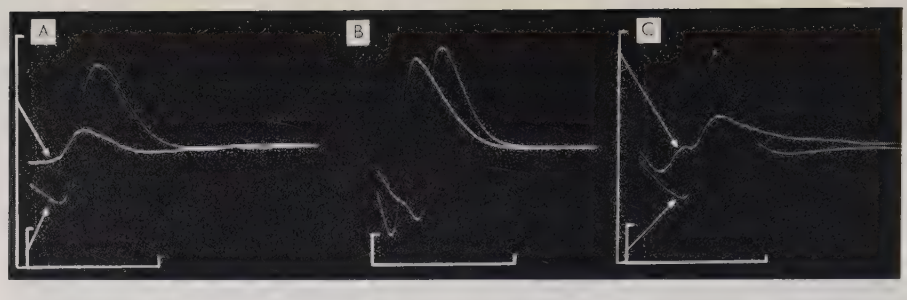

FIG. 36. *The cross-talk distortion. A* and *C* made with the 3+11 multielectrode. The fiber passes perpendicularly to the needle.

In *A* electrode 3 is aligned with the fiber (the larger spike is from electrode 3) and recording is made from electrode 13 (small spike). The latter must be caused almost entirely by capacitive cross-talk from electrode 3 since it is equal to the potentials from electrodes 12 and 11 (not shown).

In *C* the fiber is aligned with electrodes 1 and 8. From both of these there is a capacitive cross-talk to electrode 3 (the complex small potential). The large potential is from electrode 1. Propagation was in the direction from 8 to 1.

B is obtained from electrodes 2 (first potential) and 10 with the fiber aligned with these electrodes of the 3×4 multielectrode. The deformation of the initial positive phase in the second spike is caused by cross-talk. Calibration: 2 mV and 500 μsec.

bent it was very difficult to obtain action potentials of high amplitude, presumably because the fibers lying close to the electrode were damaged. The appearance of the action potential from a *partially* damaged fiber cannot be decided at present.

Serious consideration must be given to the question of what happens when a great many fibers are crushed in the vicinity of the fiber from which recording is being made. It is reasonable to assume that the intracellular fluid, rich in potassium ions, pressed out from the damaged fibers changes the composition of the extracellular fluid. This might alter the action potential either by affecting the membrane potential or by altering the volume conductor in which the action currents are distributed. No satisfactory answer can, however, be obtained at present.

The muscle as a volume conductor

This subject has been treated by BURKHARDT (1957).

The action currents from an isolated single muscle fiber immersed in Ringer solution (e.g. HÅKANSSON, 1956, 1957 *a, b*) are distributed in a volume conductor that is homogeneous (all parts of it having the same conductivity), isotropic (the conductivity being the same in all directions) and does not contain any non-linear elements (i.e. elements that do not obey Ohm's law). Furthermore, the conductivity is purely resistive.

The action currents from a muscle fiber *in situ* are distributed in a volume conductor that is inhomogeneous, anisotropic and contains non-linear elements. The conductivity is determined both by resistive and reactive components.

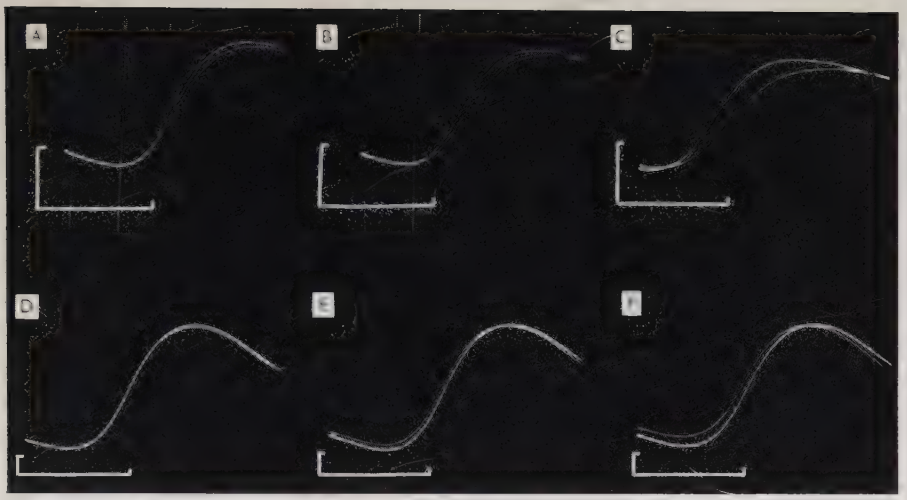

FIG. 37. *The effects of limiting the pass-band of the amplifiers.* Recordings from electrode 8 of the 3 + 11 multielectrode. Different potentials in the two rows. The larger potentials in all recordings obtained with a pass-band for electrodes and amplifiers together of 1–35,000 cps. Pass-band for the smaller potential in *A* 1–20,000 cps; *B* 1–12,000 cps; *C* 1–9,000 cps; *D* 5–35,000 cps; *E* 60–35,000 cps; *F* 140–35,000 cps. Calibration: 2 mV and 100 μsec.

Inhomogeneity. The electrical properties of the muscle fibers differ from those of the extracellular space (BUCHTHAL *et al.*, 1955 *b*) and the muscle contains fibrous tissue, fat, nerves and blood vessels. The smooth, clean shape of the action potentials from the 11 electrodes and the symmetry of the decrement curves (Fig. 34) suggest that the degree of distortion due to inhomogeneity is, as a rule, small.

Anisotropy. In the direction of the fiber axis the conductivity is mainly resistive, while in the transverse direction both resistive and reactive (caused by capacitors) (BURKHARDT, 1957). What effect this may have on the recordings cannot be decided at present.

Non-linear elements. See page 43, where the opinion of BURKHARDT (1957) was quoted, that the non-linear properties of the muscle are normally of minor importance.

The amplifiers

The distortions of the shape of the spike when the pass-band of the recording system is limited are shown in Fig. 37. Some examples of the influence of the low frequency response on the terminal phases are shown in Fig. 31. It seems that the pass-band 5–35,000 cps (for the electrode and amplifier together) gives a reasonably faithful reproduction of the action potential waveform. However, a high-pass filter of 60 cps has usually been used for all recordings (unless otherwise indicated) and this must have decreased the amplitude of the positive phase and reduced the terminal phases.

It can be calculated from the rise-time of the electrode and amplifier (12 μsec, page 29) that an original rise-time of 70 μsec increases to 71.2 μsec (i.e. an increase of 1.5 per cent) upon recording (calculated by vector addition of the two rise-times, see TERMAN & PETTIT, 1952, page 259).

Summary of the distorting factors in the present methods

1. The insulating wall around the electrodes increases the action potential voltage by a factor of up to two without any other effect on the action potential shape.

2. The metallic needle shaft does not distort the potentials appreciably.

3. The electrode area of 30 μ increases the rise-time by approximately the propagation time across the electrode (10 μsec for a fiber of velocity 3.5 meter/sec) and decreases the voltage, presumably only to a minor degree. The exact influence of the electrode area is not yet definitely established.

4. There is a capacitive cross-talk between the electrode leads that distorts the potentials. The cross-talked signal may even exceed in amplitude the volume-conducted action potential.

5. The needle may damage the muscle fiber under study. By crushing adjacent fibers the natural environment of the fiber used for recording may also be altered. The importance of these factors cannot be settled at present.

6. Inhomogeneity, anisotropy, non-linearity and the presence of reactive components in the muscle may affect the current distribution in the muscle.

7. A limited frequency response of the recording apparatus distorts the recordings. By the use of a high-pass filter of 60 cps the positive phase and the terminal phases are decreased. The high frequency response of 35,000 cps increases a rise-time of 70 msec by 1.5 per cent.

If the action potentials are used only as "time markings" to indicate *when* a certain event occurred, the distortion may be unimportant. This is, for example, the case when studying the time variations between the components of a composite potential (the jitter phenomenon, page 48), or in the determination of the propagation velocity (EKSTEDT & STÅLBERG, 1963). Determination of the direction of the propagation for delineating the end-plate zone (page 32) can be made with highly distorted action potentials. A distortion is of little importance when using the action potentials to locate active fibers.

However, if the action potential waveform shall be used for drawing conclusions about the properties of the muscle fiber, then the first step must be to determine the distortions introduced by the recording methods in order to be able to deduce the real potential variations that should occur if the recording electrode had been absent.

Parameters of the single muscle fiber action potentials

The action potentials from an isolated frog muscle fiber immersed in a large volume of Ringer solution, was maximally 2 mV when recording just outside the fiber (HÅKANSSON, 1957 *a*). BUCHTHAL & STEN-KNUDSEN (1959) recorded extracellular action potentials with a glass capillary electrode close to the surface of deep frog muscle fibers *in situ* and found the action potential voltage to be 6–7 mV. The number of measurements are not given. The larger action potential voltage obtained when recording from a fiber in situ than when immersed in Ringer solution was explained as an effect of the lower external conductivity in situ.

The maximum value of 25 mV found in this investigation may be astonishing. However, due to the "wall effect" the voltage has been increased by a factor of two and ought, in fact, to have been 12.5 mV if recorded in an unlimited volume conductor. Action potentials of such high amplitude were only exceptionally encountered and must have occurred with the electrode in immediate contact with a fiber of very large diameter. It is not likely that the high voltage recordings were intracellular recordings, because such a recording ought to be mainly a positive spike and the high voltage potentials had essentially the same shape as the low voltage potentials. 94 per cent of the recordings were below 15 mV (i.e. 7.5 mV when correcting for the "wall effect") and this seems to be in good accordance with the findings of BUCHTHAL & STEN-KNUDSEN (1959).

The spike potentials recorded in human muscle by BUCHTHAL *et al.* (1957 *a*) using a multielectrode were up to 10 mV in amplitude, and these appear to be the largest muscle action potentials so far reported.

There is a considerable discrepancy between the voltage distribution in this study and that reported for *motor unit potentials* recorded after random insertion of concentric needle electrodes by BUCHTHAL *et al.* (1954 *a*). In that study of 1268 motor unit potentials about 25 (2 per cent) were above 700 μV and none seemed to have been above 1 mV. In the present investigation two spikes out of 300 were below 1 mV while none were below 700 μV. A part of the discrepancy can be explained by the reduced distortion introduced by the present electrodes due to their smaller diameter (see Chapter 5). The remaining difference may, however, be explained in the following way: the action potentials *selected* for study in the present investigation were of a type that were only quite exceptionally encountered when recording by *randomly* inserted electrodes. In the present study an amplifier sensitivity of 100 μV/mm on the oscilloscope screen was used in seeking for the action potentials and, therefore, those below 500 μV were neglected. No doubt this has introduced a bias in the sampling of the action

potentials that must, at least in part, be responsible for the skewness in the voltage distribution curve in Fig. 32 *A*.

In previous investigations potentials of such fast time courses as found in the present investigation seem to have been only exceptionally encountered in limb muscles but commonly in the facial muscles (e.g. PETERSÉN & KUGELBERG, 1949; JASPER & BALLEM, 1949). The small distortion in the present methods explains part of this finding, but it seems reasonable to assume that also here the *selection* of a special type of action potential is responsible for the apparent discrepancy.

CHAPTER 5

SINGLE MUSCLE FIBER ACTION POTENTIALS RECORDED WITH CONCENTRIC NEEDLE ELECTRODES

In Chapter 3 (page 69) it was shown that action potentials free from jitter when recorded with the needle multielectrode were derived from single muscle fibers. The question to be answered in this chapter is whether or not this is also true when recording with concentric needle electrodes.

Methods

The concentric needle electrodes are described on page 20. The tips were ground under the microscope before each experiment. Recording was made bipolarily between the center electrode (area 100×380 μ) and the shaft of the needle. Recording has also been made between the center electrode and a subcutaneous reference electrode (page 20).

The amplifiers and the action potential display were the same as described in Chapter 1 (page 20 ff.). The oscilloscope sweeps were thus triggered by the action potentials.

The concentric needle electrodes were inserted with the shaft perpendicular to the fiber direction and approximately midway between the end-plate zone and the tendon. Two experiments were performed in the short head of the brachial biceps muscle of two healthy young men and about 400 potential recordings were made.

Results

The action potentials obtained during slight or moderate contraction after *random insertion* of the needle electrodes were essentially similar to those obtained with the same type of electrode by BUCHTHAL *et al.* (1954 *a, b*) and BUCHTHAL (1957), and to those obtained with a somewhat different type of concentric needle electrode by PETERSÉN & KUGELBERG (1949).

In the action potentials, exceeding 0.5 mV, obtained after the adjustment of the needle electrode for optimum voltage, individual spike components (cf. page 46) were usually discerned (Fig. 38). It was considerably more

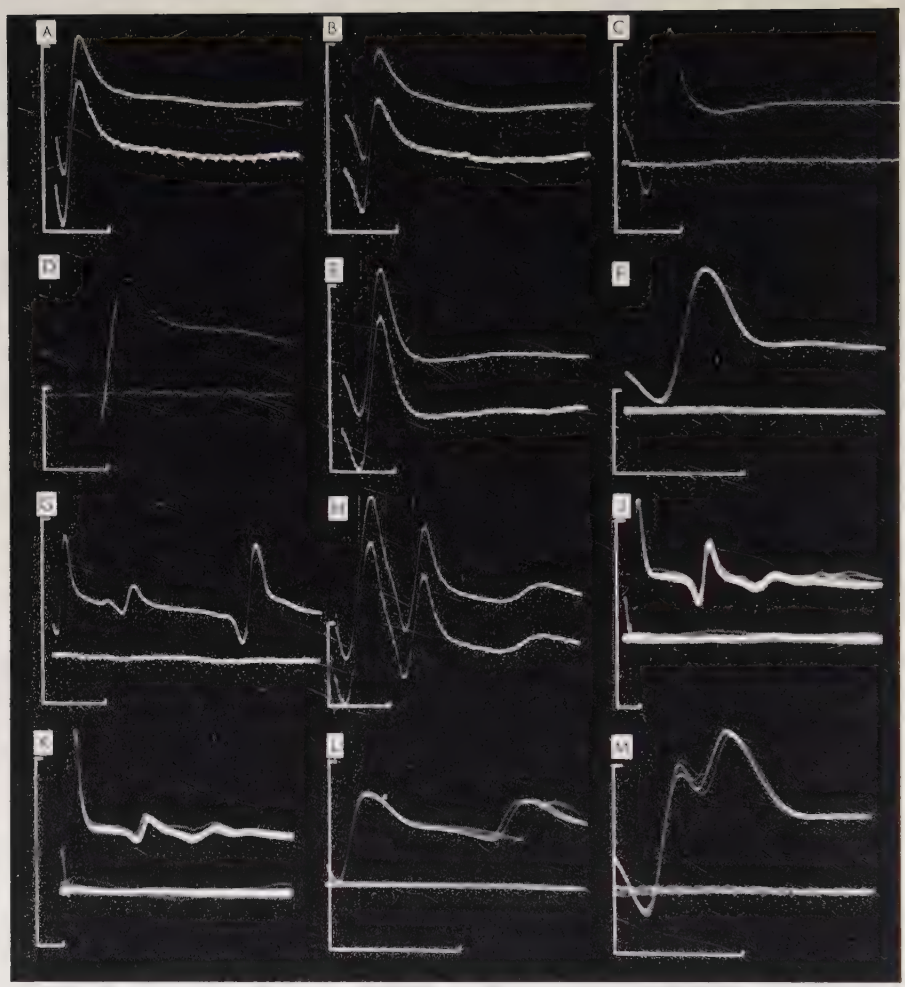

FIG. 38. *Recordings with concentric needle electrodes.* In all recordings the upper potentials are led off between the central electrode and the shaft of the needle. In *A, B, E, H* the lower potentials are led off between the central electrode and a subcutaneous reference. In *F* about 15 potentials are superimposed, in *J* about 12, in *K* about 8, in *L* about 7 and in *M* about 8. *D* has been retouched. Calibration: 2 mV and 500 μsec.

difficult to obtain isolated spike components with this electrode than with the multielectrode. In the composite potentials above 0.5 mV the *jitter phenomenon* between the component spikes was invariably seen and was of essentially the same appearance and size as when recording with the multielectrodes (Fig. 38 *J, K, L, M*). *The individual spikes were of identical shape*

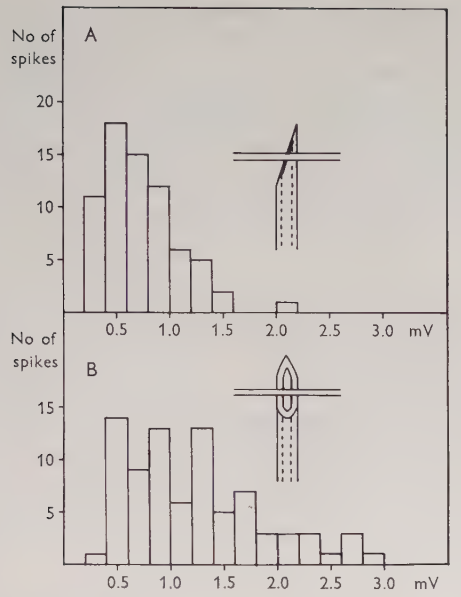

FIG. 39. *Single muscle fiber spikes from the concentric needle electrodes.* The insets show the electrode position in relation to the fiber. In each case the action potentials have been adjusted to maximum voltage by to-and-fro movements of the needle. *A,* Arithmetic mean 0.7 mV. Median value 0.7 mV. Range 0.2–2.1 mV. $N = 70$. *B,* Arithmetic mean 1.2 mV. Median value 1.2 mV. Range 0.2–2.9 mV. $N = 83$.

at consecutive discharges (Fig. 38 *F*). Their shape was similar to the single muscle fiber action potentials recorded with the multielectrodes (Chapters 2 and 4).

In composite potentials of voltage 100–500 μV individual spike components could usually be discerned, often, however, only as humps or other irregularities in the waveform. The jitter phenomenon was *usually* seen but the slow time course of the potentials could make the triggering of the sweeps unstable. The whole oscilloscopic picture was then "blurred" due to technical reasons and it was then difficult to detect the jitter. For this reason the jitter phenomenon was only rarely seen in action potentials of voltage less than 100 μV. In other words: The jitter was seen in all cases where the recording system permitted a detection of a variability in the waveform of an order of magnitude of 10 μsec (cf. page 69).

It was far more difficult to keep the electrode in constant position in relation to the fiber when using concentric needle electrodes than when using electrodes at the side of a needle, and movement artefacts were then more liable to obscure the jitter phenomenon.

In 83 recordings the needle position was adjusted for optimum voltage of the recorded spike potential. In all these recordings the optimum position proved to be that in which the electrode in the bevel surface of the tip faced the fibers. The voltage distribution of these spikes is given in Fig. 39 *B*.

In another series of experiments the shaft of the needle was turned 90° as shown in Fig. 39 *A* and kept so during the search for potentials. The adjustment for optimum voltage was then made only by to-and-fro adjustments of the needle. Fig. 39 *A* shows the voltage distribution obtained for 70 spikes.

The rise-time for the spikes was of the order of 200 μsec and the spike duration (measured according to Fig. 7) of the order of 500–1000 μsec, both parameters approximately twice as large as with a 30 μ electrode. No statistical analysis of the influence of the needle position on the time course of the potentials has been made in this investigation.

There seemed to be no substantial difference between the recordings in which the shaft of the needle was used as reference electrode and those in which separate subcutaneous reference was used.

Discussion

As the jitter phenomenon is as clearly visible when recording with concentric needle electrodes as with the multielectrodes, it may be stated that action potentials recorded with the former and free of jitter are single muscle fiber action potentials, provided that in each individual case the recording system permits the detection of a variability in the waveform of the order of 10 μsec.

These jitter-free spike potentials seem to be the same sort of potentials as described by BUCHTHAL *et al.* (1957 *a*) as "diphasic components of short duration and high amplitude" and termed by them *"the spike of the motor unit potential"*.

The lower voltage and slower time course of the single muscle fiber action potentials recorded with the concentric needle electrodes must be explained as an effect of the greater electrode area (cf. page 74) but it is possible that the part of the shaft of the needle that surrounds the bevelled surface of the electrode is partly responsible. It might be more difficult to come close to an active fiber with the electrode in the bevel surface than with the electrode at the side of the needle.

The larger electrode area in the concentric needle electrodes also explains why single muscle fiber action potentials were more rarely encountered than when using the multielectrode. With increase of the electrode area the chance

of there being more than one fiber belonging to a particular motor unit close to the electrode will increase.

The increased voltage of the spike potentials when the bevel surface of the needle *faces* the fibers in comparison to when the needle is rotated 90° from this position is to be expected, but it does not seem to have been taken into account in previous investigations.

CHAPTER 6

SOME COMMENTS ON THE FIBER ARRANGEMENT OF THE MOTOR UNIT

BUCHTHAL *et al.* (1957 *a*) consider the fibers of the motor unit to be arranged in "subunits", i.e. groups of 10–30 adjacent fibers. The individual fiber potentials within the subunit are considered to be synchronized and to give rise to a smooth biphasic spike potential, called "the spike of the motor unit potential". Their experimental evidence is summarized on page 11 ff.

With the fibers of a motor unit arranged in subunits of diameters 150–300 μ, as described by BUCHTHAL *et al.*, it ought to be a common experience to pierce a subunit with the needle.

When using the multielectrode, synchronous spike potentials of high voltage should then be obtained from several of the electrodes. When using the "Janus electrode" (Fig. 3, page 19), which has two electrodes mounted on opposite sides of a needle, synchronous action potentials ought to be recorded from the two electrodes.

Methods

Recordings with the 3+11 multielectrode.

These recordings are from the same material, and made with the same methods, as described in Chapter 3.

Recordings with the "Janus electrode".

The Janus electrode (page 19 and Fig. 3) was used in five experiments in the biceps muscles of three young men who did not have any signs of neuromuscular disorder. Single muscle fiber action potentials (defined as spike potentials of identical shape at consecutive discharges, page 69 were sought on one of the electrodes, which was then adjusted for optimum position in relation to the fiber. Recording was then made from the electrode at the other side of the needle, and 100 such recordings were made.

Results

The 3+11 multielectrode

In the several thousand potential recordings that have been made, action potentials of approximately the same high voltage *and* synchronous to

within 50 μsec (cf. page 47) were never found on three, four or five adjacent electrodes (corresponding to a distance of 150–300 μ). Two adjacent fibers could sometimes have nearly simultaneous action potentials, which was easily revealed by the jitter phenomenon and by the bipolar recording (page 63). Complex, "polyphasic" potentials were occasionally generated by groups of up to 11 fibers, as revealed by the recordings from the different electrodes in the long row. These groups were not usually homogeneous but also contained fibers from other motor units. The jitter phenomenon was invariably present in a composite potential.

The "Janus electrode"

In 48 of the recordings a spike potential was found only on the electrode that was mounted opposite to the bevel surface, and in 44 only on the electrode mounted at the same side as the bevel surface. In 8 recordings spike potentials were found on both electrodes at the same time. The jitter phenomenon was then invariably present between the two spikes. The voltage distribution was about the same for the spikes obtained from the two electrodes.

Discussion

The subunit of BUCHTHAL *et al.* (1957 *a*) is considered to be a functional and not a structural entity. Even if there was a surrounding sheath, there should have been a considerable chance of piercing the subunits. The fact that no recording indicating such a piercing was encountered is inconsistent with the subunit concept.

It might be argued that in the recordings with the Janus electrode the fibers pushed aside by the bevelled tip might have become damaged, and that even if a subunit had been pierced, spike potentials would be found only on one side of the needle. However, the voltage distribution was equal for the spikes from both sides of the needle and the spikes were found equally often on both of them. Thus, fiber damage could not have been extensive and also the results with the Janus electrode are inconsistent with the subunit concept.

In fact, the subunit potentials seem to be the same type of potentials as those that are considered to be generated by single muscle fibers in this investigation.

BUCHTHAL *et al.* (1957 *a*) claim that there is a mechanism of mutual interaction between adjacent muscle fibers that synchronizes the propagation of the action potentials. This synchronization was assumed in order to

explain the smooth shape of the subunit spike potential generated by 10–30 muscle fibers with different diameters and different sites of innervation.

It is true that with *perfect* synchrony between the individual fibers most of the single muscle fiber criteria would be fulfilled by a subunit, and this subunit should then behave mainly like a single very large fiber. However, the not infrequent findings of action potential sources with center-to-center distances of the order of 60 μ and nevertheless showing jitter (page 57, Fig. 17 *A, C, J, L, M*) or distinctly different propagation velocities (page 47, Fig. 16 *A, B*) cast doubt on the existence of a synchronization mechanism and are not compatible with the subunit concept.

An attempt will be made to explain how BUCHTHAL *et al.* have been forced to assume the concept of the subunit. It would seem that the crucial point is that they have overlooked the cross-talk between the electrodes (page 75). This alters the relation between the spike voltage and the distance along the electrode array in the multielectrode, which is the basis for their volume conduction analysis from which they have calculated the size of the spike source.

The present data give little positive information concerning the fiber arrangement of the motor units. They are, however, consistent with the findings of several authors in animal muscle (see page 10 ff.) that there is intermingling of the muscle fibers belonging to different motor units.

SUMMARY

The present investigation is a study of single muscle fiber action potentials in the normal human electromyogram. The possibility of recording such potentials has been questioned or denied. Action potentials of seemingly simple shape have often been recorded, but there has been no way to decide whether they were derived from single fibers or from aggregates of fibers. However, with the knowledge gained from the present investigation it is now possible to identify and study those potentials that are derived from single muscle fibers.

In *Chapter 1* the methods are described. Most experiments were made with a needle multielectrode with fourteen 30 μ platinum electrodes mounted flush with the side of the needle. The small electrodes permitted recording with but little distortion; the electrode arrangement made possible recording from several sites simultaneously. The action potentials were displayed on the oscilloscope with the sweep triggered by the action potential.

To define the characteristics of a single muscle fiber recording made with the multielectrode, a method was developed (*Chapter 2*) for chemical activation of single muscle fibers *in situ* by micro-injection of sodium citrate into the muscle. There are strong reasons to believe that muscle fibers chemically activated in this way are independently discharging. The most important property of these single fiber recordings was that all consecutive discharges were of identical shape.

In the voluntarily activated muscle the fibers are not independently discharging. The question then arises whether it is possible for two or more adjacent fibers to generate composite potentials that simulate those from a single fiber. This question is dealt with in *Chapter 3*, where a study has been made of *obviously composite* potentials in order to gain experience in what is to be expected from composite potentials where the composite character is not detected in the shape. Some quantitative aspects of this

problem were also treated by "synthetic composite potentials" obtained by adding simple potentials of known shape, voltage and mutual time interval. The most important finding was that composite potentials were never of equal shape at consecutive discharges. There were small variations in the waveform, the *jitter phenomenon*. The cause of the jitter was found to be a variability of the order of 10–30 μsec in the mutual time interval between the spike components of the composite potentials. The individual spike components were, however, of identical shape at consecutive discharges. The jitter phenomenon was interpreted as being caused by a variability in the end-plate delay of the individual muscle fibers.

It was found that spike potentials that were free of jitter behaved like the chemically activated ones in all other respects as well and that such potentials could not have been generated by two or more adjacent fibers (all making appreciable contributions to the potential form). An interference from a remote spike exceeding 10 per cent of the amplitude of the main spike would also be detected by the jitter phenomenon. Thus, *spike potentials that are free from jitter are single muscle fiber action potentials provided the time resolution in the recording system is such as to permit the detection of a variability in the waveform of the order of 10 μsec.*

In *Chapter 4* some parameters of the single muscle fiber action potentials are reported. The action potentials had a clean, smooth biphasic spike component, often followed by terminal phases of low amplitude and long duration. The median value for the voltage was 5.6 mV and the maximum value obtained was 25.2 mV. The median value for the spike duration was 470 μsec. These values are not representative for the total fiber population since the method favours the recording from larger fibers.

In *Chapter 5* it is shown that single muscle fiber action potentials can also be recorded with concentric needle electrodes and identified by the absence of jitter.

Buchthal, Guld & Rosenfalck have concluded that the clean smooth spikes obtained during voluntary contraction are derived, as a rule, from small groups of perfectly synchronized, tightly packed fibers, "subunits". In *Chapter 6* results are presented which are not in accordance with the subunit concept. Recordings consistent with a piercing of the subunit by the multielectrode were never obtained. Non-simultaneous action potentials from closely adjacent fibers belonging to the same motor unit were, however, found.

The present findings indicate intermingling of fibers belonging to different motor units.

ACKNOWLEDGEMENTS

This work was carried out in the Department of Pharmacology, University of Uppsala. To the head of the department, Professor Ernst Bárány, I express my sincere gratitude for his stimulating advice and for his penetrating and constructive criticism which has been of inestimable value.

The experimental work and theoretical analysis have been made in close collaboration with Dr. Erik Stålberg. I wish to thank him most sincerely for his constant, unselfish help.

Docent Karl-Erik Hagbarth, Docent Lars-Erik Larsson and Docent Sigfrid Blom have read the manuscript and offered important suggestions. For this and for valuable discussions during the course of the work I am greatly indebted.

This work could not have been performed without the skilful assistance of the staff of the department. I am particularly grateful to Research Engineer C. Iwar Sjöberg for construction of many of the electronic devices; to Mr. Per Häggqvist for technical assistance, not least in the construction of the needle electrodes; to Mr. Manne Fredriksson for help with photographic work and in many other respects; to Mr. Leif Andersson for technical assistance; to Mrs. Ann-Mari Kjellberg and Mrs. Kerstin Sundström for secretarial aid; to Mr. Torsten Ekwall for most of the drawings in the figures and to Mr. Folke Högberg for help with some mechanical constructions.

My thanks are also due to all those who have acted as experimental subjects.

I am most indebted to my wife Elsi without whose continued encouragement and aid this work would not have been completed.

Financial support has been provided by the Medical Faculty of Uppsala, the Svenska Personal-Pensionskassan, the Magnus Bergwalls Stiftelse, the Riksföreningen mot Polio and the Anna Maria Lundins Stipendiefond, and to all these I express my gratitude.

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